Are scrapie-susceptible sheep more productive?

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Thesis submitted for the degree of Doctor of Philosophy

The University of Edinburgh
2006

Declaration

I hereby declare that the work presented in this thesis is my own, except where stated. No part of this work has been, or will be submitted for any other degree or professional qualification.

Chapter 3 has been published in the following journal:

Nicholls, N., Kruuk, L., Woolhouse, M., Stevenson, E., Gravenor, M., Baylis, M. (2006) Do farmers have a higher regard for scrapie-susceptible sheep? *Veterinary Record* **158,** 732-734.

Acknowledgements

I would like to thank everyone who has been involved in the studies contained in this thesis. All my supervisors, past and present, who have helped me put this together: Mike Gravenor and Loeske Kruuk for their statistical advice, Matthew Baylis and Mark Woolhouse who have been my main supports and sources of advice over the years, and more recently Simon Gubbins, who stepped in at a late stage, and has still contributed very useful advice in the finishing stages.

I would also like to thank all the farmers who put up with me on their farms, taking the time out of their already busy schedules to help me with the various studies in my project, and their patience when things did not always go according to plan. I am also grateful to Emily Stevenson, for all her genotyping work, Delia Jones for data entry and general assistance, and to Charles Chihota, Chris Trower for their assistance on the farms. I am also grateful to Sam Boon at the Meat and Livestock Commission for the provision of the Signet Sheepbreeder® data, and Margo-Chase Topping and Mick Crawley for analytical advice, on survival data and time series data respectively.

To my father Harry, and other family and friends who have all been supportive of me over the past few years, and to Vicky, for a city girl's help on a farm visit. Finally to Sarah, who has had to really put up with my stress over the past few months. Thank you.

This thesis is dedicated to the memory of my mother Paulette, may she rest in peace.

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Abstract

A relationship between scrapie susceptibility, which is determined by *PrP* genotype, and valuable production traits has long been noted by sheep farmers, with many claiming that their 'best' sheep often are found to be susceptible to scrapie, or have close siblings that are susceptible to scrapie. There have been several historical anecdotal reports to support this observation, but only recently has the hypothesis, that scrapie-susceptible sheep are more productive, been investigated in scientific study. This thesis contains the results of several such studies and is concluded by an investigation into whether the current breeding strategies being encouraged in the UK would be effective at eliminating scrapie from the national sheep flock. In these studies, *PrP* genotype was compared to objective measures such as lamb weights and Estimating Breeding Values (EBVs), as well as to subjective measurements which were based on a farmer's judgement of their sheep, with varying results. Analysis of the subjective measurements, rating scores and culling records did not show any association with *PrP* genotype. The results of the analysis of the EBVs were variable, and inconsistent between farms, with susceptibility to scrapie being associated with both increased and decreased productivity. There was a small association between *PrP* genotype and lamb weights, which indicated that at eight weeks of age, ARR/ARR lambs were slightly smaller than lambs of other more susceptible genotypes. Overall, however, there is no strong evidence that scrapie-susceptible sheep truly are more productive. The final section has shown that with the current suggested breeding strategies, there will still be some risk of scrapie outbreaks in some flocks.

1 General Introduction

1.1 Background information

It has long been claimed by farmers that 'scrapie-susceptible sheep are more productive' and past anecdotal reports have implied that is the case: 'superior' show sheep have appeared to succumb to scrapie more often (Parry, 1962); and lambs with superior liveweight gain which carried the scrapie susceptibility trait have been reported (Steele, 1964). However, this theory, that scrapie-susceptible sheep are indeed more productive, had not been tested in a large-scale study prior to 2002 and it is the focus of this thesis, which examines this theory by comparing various subjective and objective productivity parameters in sheep to their *PrP* genotype (see section 1.3).

Scrapie is a fatal infectious neurodegenerative disease of sheep which is known to have been present in Europe from the early 1700s (Parry, 1962; Parry, 1983a). It is a member of the Transmissible Spongiform Encephalopathy (TSE) group of diseases, which includes Bovine Spongiform Encephalopathy (BSE) in cattle, Transmissible Mink Encephalopathy; and in humans, Kuru and variant Creutzfeldt-Jacob Disease (vCJD).

The exact nature of the infectious agent which causes scrapie is yet to be confirmed (Somerville, 2000; Detwiler and Baylis, 2003). Currently, the most widely accepted theory on the pathogenesis of scrapie is the 'prion hypothesis', that is that this agent (prion), uses PrP^c, a glycoprotein normally encoded by the host *PrP* gene, as a

template to produce more of itself: a protease-resistant infectious isoform, PrP^{Sc} (Prusiner, 1982; Prusiner, 1998). PrP^{c} is normally broken down by proteases, but as PrP^{Sc} is protease-resistant, it accumulates in the brain and other tissues and disrupts the normal cellular structure. The central role of PrP^{c} as a template for the production of PrP^{Sc} is supported by numerous studies (Büeler *et al.*, 1993; Sailer *et al.*, 1994; Weissmann *et al.*, 1994a; Weissmann *et al.*, 1994b). For example, it has been shown that mice which cannot produce PrP^{c} do not develop scrapie when experimentally challenged (Büeler *et al.*, 1993; Sailer *et al.*, 1994; Weissmann *et al.*, 1994a; Weissmann *et al.*, 1994b).

1.2 Clinical signs and diagnosis of classical scrapie

Scrapie has an insidious onset with the first clinical signs usually being seen between 2-5 years of age, but sometimes earlier, with death usually occurring within 6 months of the onset of clinical signs (Parry, 1962; Parry, 1983b). The first sign of scrapie is often an altered behavioural status: the sheep may just remain staring into space, be restless, or lose its fear of humans and the sheepdog. Subsequently, the more 'classic' sign of pruritus may appear, with the sheep rubbing its rump, flanks, or poll of the head against inanimate objects. Wool loss may also occur, and often any regrowth is pigmented. As the disease progresses, disorders of gait may be seen, such as incoordination and hypermetria, with fasciculation of the muscles and generalised tremors. By this stage, weight (and muscle mass) loss is apparent, but without reduction in appetite. There may be subtle evidence of disrupted homeostasis, such as drinking more frequently, but of reduced amount. In some cases, other evidence of nervous disturbance may be present, such as epileptic fits and "dog sitting"

(posterior paralysis). Scrapie cases can become recumbent, and in some instances can be found dead.

Histopathological samples taken post-mortem from affected sheep show degeneration of the central nervous system tissues, affecting mainly the medulla, pons, midbrain and thalamus. Vacuolation, loss of neurones and swelling and degeneration of the astrocytes occurs (Detwiler, 1992) with no local inflammation or immune response. Definite confirmation of scrapie is achieved by immunohistochemical detection of PrP^{Sc} in sections from affected brain, or detection using Western Blot.

1.3 Genetic factors affecting susceptibility to scrapie

The impact of genetics on scrapie is well documented, and is a key characteristic of the disease. For many years, it was even argued that scrapie was solely a genetic disease, and not transmissible.

Dickinson *et al.* (1968) first suggested that susceptibility to scrapie was influenced by a single gene, termed the *S*crapie *i*ncubation *p*eriod (*Sip*) gene, with two alleles pA (long incubation period) and sA (short incubation period). This hypothesis was based on work done at the Institute for Animal Health (IAH) Neuropathogenesis Unit (NPU) in Edinburgh using two lines of Cheviot sheep, one bred for resistance to scrapie and the other for susceptibility, which were experimentally infected with SSBP/1 (an isolate of scrapie agent commonly used in research obtained from a pool of sheep brains). sA/sA homozygotes and the sA/pA heterozygotes were associated

with short incubation periods when infected with SSBP/1, whereas pA/pA homozygotes were associated with long incubation periods or not developing scrapie at all (Dickinson and Outram, 1988); and it was suggested that the development of both natural and experimental scrapie were both controlled by the *Sip* gene (Foster and Dickinson, 1988; Goldmann *et al.*,1990 and 1991). The importance of different strains of scrapie was also highlighted, as it was found that it was pA/pA homozygous sheep which had the shortest incubation times when challenged with scrapie isolate CH1641 (Dickinson and Outram, 1988).

A series of experiments demonstrated the connection between the two genes, *Sip* and *PrP*, and the term *Sip* is no longer used. Restricted fragment length polymorphisms of the sheep *PrP* gene were found that could act as markers for the *Sip* gene (Hunter *et al.*, 1989; Goldmann *et al.*, 1991); with similar results being demonstrated in mice (Carlson *et al.*, 1986; Hunter *et al.*, 1987; Hunter *et al.*, 1989). The *Sinc* gene, which is the mouse version of the *Sip* gene, was found to be equivalent to mouse *PrP* gene. Goldmann *et al.* (1994a) suggested that homozygosity for *Sip* pA corresponded with encoding alanine at codon 136 of the *PrP* gene, and *Sip* sA corresponded with encoding valine at that codon, as V_{136} sheep all developed scrapie after challenge with SSBP/1.

In the UK sheep breeds, polymorphisms of five amino acids (alanine (A), valine (V), arginine (R), glutamine (Q) and histidine (H)) at codons 136, 154 and 171 of the *PrP* gene are known to be important in determining susceptibility to scrapie (Belt *et al.*, 1995; Hunter *et al.*, 1996, Hunter, 1997). The polymorphisms at these positions

can potentially give rise to twelve alleles, not all of which have been reported, with only five alleles are commonly seen, $A_{136}R_{154}R_{171}$ (ARR), ARQ, VRQ, AHQ and ARH (Belt *et al.*, 1995; Ikeda *et al.*, 1995). The alleles VRR, in Nolana sheep, and AHR, in Texel, Suffolk and Nolana sheep have been reported (Kutzer *et al.*, 2002) and other allelic variations are present in non-UK sheep breeds (e.g. Acin *et al.*, 2004; Billinis *et al.*, 2004; Gombojav *et al.*, 2004).

Encoding A_{136} is associated with resistance to scrapie, whereas V_{136} is associated with susceptibility to scrapie (Laplanche *et al.*, 1993; Hunter *et al.*, 1994; Hunter *et al.*, 1996). At codon 154, encoding H has been associated with resistance to classical scrapie, with R being associated with susceptibility (Laplanche *et al.*, 1993). Encoding QQ_{171} confers susceptibility to scrapie, and homozygosity for VRQ is the genotype most susceptible to classical scrapie (Belt *et al.*, 1995; Hunter *et al.*, 1996; Hunter, 1997; Dawson *et al.*, 1998), whereas sheep that are homozygous for ARR appear to be the most resistant to developing classical scrapie (Hunter, 1997).

The scrapie strain is also important. Experimentally all QQ_{171} sheep succumbed to challenge by BSE or CH1641 scrapie (i.e. no effect of V_{136}), whereas it seemed that encoding V_{136} (hetero- or homozygous) enhanced susceptibility to SSBP/1 experimental scrapie (Goldmann *et al.*, 1994b; Hunter *et al.*, 1997). More recently, a novel strain of scrapie, Nor98, has been identified (Benestad *et al.*, 2003), which affects animals encoding the AHQ allele (Moum *et al.*, 2005); a genotype usually associated with some resistance to classical scrapie.

The breed of the sheep also influences which genotypes are susceptible to scrapie. In breeds which encode valine (e.g. Swaledales, Cheviots), the genotype ARQ/ARQ is of intermediate to high susceptibility to scrapie, but in the Suffolk breed, which does not normally encode valine, the ARQ/ARQ genotype is the most susceptible to scrapie (Hunter, 1997; Dawson *et al.*, 1998). Another contrasting example of breed differences is seen in German Merinoland Sheep. In this breed, encoding the genotype AHQ increases susceptibility to scrapie (Luhken *et al.*, 2004), whereas in most UK breeds this allele is associated with increased resistance to scrapie. Other studies have shown that in Texels, approximately 10% of scrapie cases are ARQ/ARQ (Belt *et al.*, 1995; Baylis *et al.*, 2002), and that in a flock of Romanov sheep, approximately 38% of scrapie case are ARQ/ARQ (Elsen *et al.*, 1999; Diaz *et al.*, 2005).

Other areas in the *PrP* coding region may also be associated with developing scrapie depending on the strain of scrapie and breed of sheep. For example, polymorphisms were found at positions 137, 138 and 151 in Icelandic sheep, but no association with disease could be determined from these alleles, as they were present in low frequencies, although $AC_{151}RQ$ was only found in unaffected sheep (C – cysteine, Thorgeirsdottir *et al.*, 1999). Position 112 is potentially associated with scrapie susceptibility in Mongolian sheep breeds (Gombojav *et al.*, 2004); and in another example of strain-genotype interaction differences, polymorphisms at position 141 are also associated with increased susceptibility to Nor98 scrapie. Sheep encoding phenylalanine (F) at position 141 are also at higher risk of developing scrapie (Moum *et al.*, 2005).

The original guide to aid British farmers when estimating an individual sheep's risk of developing scrapie based on these breed and genotype differences was developed by The Sheep Information Group in 1998 (Dawson *et al.*, 1998). This guide was to enable farmers to identify susceptible animals among their stock, and allow for selective breeding of resistant sheep. This guide has since been superseded by the National Scrapie Plan (NSP) classification scheme (table 1.1: this highlights the Ram Genotyping Scheme), which places more emphasis on breeding strategies.

Table 1.1 NSP classifications for the 15 *PrP* genotypes present in the UK. The third column provides information on what must happen to rams within each class. These restrictions do not apply to ewes (DEFRA, 2005).

Genotype	NSP Type	Restrictions (males only)
ARR/ARR	1	No restrictions apply
ARR/AHQ	2	
ARR/ARQ	2	
ARR/ARH	2	
AHQ/AHQ	3	Sale and breeding restrictions for type 3 rams no longer apply
ARQ/AHQ	3	
AHQ/ARH	3	
ARH/ARH	3	
ARQ/ARH	3	
ARQ/ARQ	3	
ARR/VRQ	4	Immediate restriction on sale, transfer or breeding. Slaughter or Castration required within 90 days
AHQ/VRQ	5	
ARQ/VRQ	5	
ARH/VRQ	5	
VRQ/VRQ	5	

The National Scrapie Plan for Great Britain was introduced in 2001 as a means of eliminating scrapie in the UK by breeding sheep resistant to scrapie, and potentially BSE, if it is indeed present within the national flock. The presence of BSE within the sheep flock, constitutes an increased public health risk of acquiring vCJD from

eating sheep meat products (Ferguson *et al.*, 2002), and the NSP should reduce this possible health risk. The NSP allocates sheep to different classes for breeding purposes, depending on genotype and the scrapie incidence rate of each genotype, with sheep encoding ARR/ARR being in the highest class and the most desirable for breeding purposes (table 1.1). The aim of the NSP is to reduce the number of susceptible sheep by increasing the frequency of the ARR allele to a level where scrapie cannot be maintained with the population (DEFRA, 2005b).

There are several potential drawbacks to the NSP. Recently, it has been shown that sheep encoding ARR/ARR can succumb to BSE after intracerebral challenge with infected cattle brain inoculate (Houston *et al.*, 2003). Although BSE in this genotype has not developed from oral challenge (Goldmann *et al.*, 1994b; Jeffrey *et al.*, 2001), which is thought to be the natural route of infection, it suggests that ARR/ARR sheep are not completely resistant to developing TSEs, as this was recently proven. Certain strains of scrapie not only infect sheep previously thought to be resistant, but the PrP^{Sc} from these sheep was infectious, and could be transmitted to mice expressing the ovine *PrP* gene by intracerebral injection (Le Dur *et al.*, 2005).

Ikeda *et al.* (1995) have reported a case of scrapie in a Suffolk sheep encoding ARR/ARR in Japan, but no other evidence of clinical scrapie in ARR/ARR sheep has been reported before or since. Another drawback to the NSP is that it does not allow for scrapie strain differences and the emergence of new disease strains. For example, the recent discovery of the Nor98 strain which, as already mentioned, targets sheep encoding AHQ. Additionally, other strains have been discovered by surveillance

which can infect ARR/ARR sheep (Buschmann *et al.*, 2004; Orge *et al.*, 2004), although no clinical disease has yet been seen in sheep of this genotype.

Another potential drawback of the NSP is an increased incidence of inbreeding, from using a reduced pool of rams to sire flocks, and the concurrent effect inbreeding will have on the productivity and overall fitness of the sheep. In some breeds, such as the North Ronaldsay, Soay and Hill Radnors, using only rams encoding ARR/ARR could also result in the extinction of those breeds, as the frequency of resistant genotypes is so low (Townsend *et al.*, 2005).

1.4 Scrapie infectivity and transmission

Previously, scrapie has been claimed to be a solely genetic disease because of the important role of genetics in scrapie susceptibility, and the fact that the disease tended to run in family lines. This claim has been disproved by several studies which have shown the infectious nature of the disease.

The first demonstration of this was in the 1930s, when a study of eight sheep showed that five of these developed experimental scrapie after inoculation with infectious nervous tissue, and that previously healthy sheep (and a goat) could develop scrapie after exposure to diseased animals (Cuillé and Chelle, 1939). In addition, it was seen that scrapie could spread to sheep believed to be free of the disease and goats by contact with affected animals (Brotherston *et al.*, 1968). Conversely, a group of susceptible sheep kept in isolation did not develop scrapie, unlike their exposed counterparts (Dickinson *et al.*, 1974).

Sheep from Australia and New Zealand (widely assumed to be scrapie-free countries) have been genotyped, and within the population are sheep which are susceptible to scrapie, yet there have not been any known cases of disease (Hunter *et al.*, 1997). Susceptible sheep imported from these two countries did not succumb to scrapie if reared in a clean (i.e. one that is not contaminated with scrapie) environment (Hunter and Cairns, 1998), but can still develop scrapie if challenged (Houston *et al.*, 2002), which suggests that scrapie is not solely genetic in nature, and that there must be exposure to infection before disease can develop.

The transmissible nature of scrapie was dramatically demonstrated in the UK in the 1930s. A vaccine against louping ill, a tick-borne virus of sheep, was produced using tissues (including brain and spleen) of sheep, including some from scrapie-infected flocks. The tissues were treated with formalin to kill viruses and bacteria, but unknown at the time, the scrapie agent is resistant to formalin. Around 16,000 sheep were vaccinated, and 35% then went on to succumb to scrapie within 2 – 3 years. To confirm the role of the vaccine, 788 sheep were injected with tissues from scrapie-infected animals. Of these sheep, 60% of those which had received intracerebral injections, and 30% of those which had received subcutaneous injections developed scrapie, which confirmed that the vaccine made from infected tissues had caused the scrapie epidemic (Ridley and Baker, 1998). More recently, a scrapie outbreak among sheep and goats in Italy has been described (Caramelli *et al.*, 2001). This outbreak occurred over the years 1997 – 1998, and was attributed to the use of a vaccine against *Mycoplasma agalactiae*.

The natural routes of transmission are unresolved. Past analysis of available data on transmission between sheep has shown that most transmission within a flock is horizontal, between unrelated flock mates (Hoinville, 1996), with limited vertical transmission. Lambs which were removed from their scrapie-affected mothers at birth still developed scrapie (Hourrigan and Klingsporn, 1996), however, the authors were not sure if it was true vertical transmission, or whether it was exposure during birth, or imperfect isolation from infection in the environment. PrP^{Sc} has been detected in the reproduction organs of female sheep (uterus, caruncle, cotyledon, and ovaries) and amniotic fluids (Hourrigan and Klingsporn, 1996), and in the placenta of sheep (Ikegami *et al.*, 1991; Andreoletti *et al.*, 2002). Foote *et al.* (1993) found that embryo transfer can prevent the transmission of scrapie from dam to offspring, although other work has not found this to be the case, and that scrapie in the lambs was unaffected by embryo transfer (Hunter, 2003, pers. comm.).

Further evidence that scrapie transmission does not only occur vertically is that studies have not shown any evidence of PrP^{Sc} accumulation in foetal tissues as late as 140 days gestation (Andreoletti *et al.*, 2002). The genotype of the lamb also seems to influence accumulation of PrP^{Sc} in the placenta, as it was observed that ewes subclinically infected with scrapie mated with resistant rams (ARR/ARR) did not accumulate PrP^{Sc} in the placenta (Andreoletti *et al.*, 2002). Furthermore, scrapie-affected Suffolk and Suffolk x Hampshire ewes accumulated PrP^{Sc} in the placenta if they were carrying a lamb with a susceptible genotype (QQ_{171}), but not if the lamb

encoded a resistant genotype (QR_{171}; Tuo *et al.*, 2002). These studies suggest that scrapie may be acquired perinatally rather than prenatally.

Scrapie also can spread horizontally to goats if reared in contact with scrapie-affected sheep (Brotherston *et al.*, 1968; Hourrigan *et al.*, 1979; Toumazos, 1991). Infection of sheep and goats is most likely to occur via the oral route (Andreoletti *et al.*, 2000), although it is also possible to transmit scrapie infection via the conjunctiva and through breaks in the skin (Stamp *et al.*, 1959; Haralamb.H *et al.*, 1973; Taylor *et al.*, 1996). For oral transmission to occur, the scrapie agent must be released from the diseased animal, whether actively secreted by the body, or excreted. As mentioned above, the placenta from scrapie-infected ewes is a source of PrP^{Sc} (depending on the lamb genotype), and infected placenta can cause scrapie in animals if fed orally, and thus is a major source of infection and contamination of the environment (Pattison *et al.*, 1972; 1974; Race *et al.*, 1998), which may account for the higher risk of scrapie on farms which practice indoor or group lambing (McLean *et al.*, 1999). Additionally, the nature of the scrapie agent allows it to survive in the environment for several years (Brown and Gajdusek, 1991), so naïve sheep may be indirectly exposed to infection if introduced to a area which has contained scrapie-infected sheep previously.

It is possible that sheep of certain genotypes may not be resistant to scrapie, but can be subclinically infected without developing clinical disease within their lifespan. This might be the case in the previously mentioned atypical cases of scrapie, where ARR/ARR sheep were found to have PrP^{Sc} accumulation within the brain tissue, but

no clinical signs (Buschmann *et al.*, 2004; Orge *et al.*, 2004). Hill *et al.* (2000) demonstrated subclinical infections in mice, where in the absence of clinical disease, there were large accumulations of PrP^{Sc} within the brain and the infection was transmissible. It is not certain if these phenomena could occur naturally in sheep. If so, 'resistant' sheep would in effect be carriers, which may make it harder to eliminate the scrapie agent (Woolhouse *et al.*, 1998). However, peripheral deposits of PrP^{Sc} have not been found in clinically normal ARR/ARR sheep on scrapie-affected farms; nor in sheep challenged orally with scrapie or BSE; nor in ARR/ARR sheep which have succumbed to scrapie following intracerebral challenge suggesting that these sheep may not be naturally infectious (Jeffrey *et al.*, 2001; Houston *et al.*, 2003).

1.5 Persistence of the VRQ allele in the UK

Natural selection is expected to remove deleterious alleles. The VRQ allele certainly appears to be deleterious in scrapie-affected flocks, so why does this allele and the disease scrapie persist in the UK national flock?

One reason may be that the scrapie epidemic in the UK is ongoing, on a long time scale, but the VRQ allele is slowly being selected out, and scrapie may ultimately die out (Woolhouse *et al.*, 2001; Gubbins, 2005). The selection pressure on the VRQ allele is unlikely to be very high, as the actual incidence of the disease is very low. In a postal survey conducted in 1998, only 2.7% of farmers reported having scrapie on their farm within last 12 months, with a median within-flock incidence of 0.37 % per year (Hoinville *et al.*, 2000), and another survey four years later saw the number of

farmers reporting scrapie on their farm over the previous 12-month period reduced to 1.0% (Sivam *et al.*, 2003). Apart from the low incidence of scrapie on most farms, the late age of onset of the disease allows for some reproduction to occur, so an affected ewe can produce 1-2 crops of lambs encoding susceptible alleles, before dying of scrapie, and susceptible rams can contribute many more susceptible lambs to the population.

Scrapie may also persist in the UK because of the structure of the national sheep flock. The national flock does not mix freely, so pockets of scrapie-affected flocks may remain, which can subsequently infect other susceptible flocks upon trading or mixing. Another factor contributing to the persistence of scrapie and susceptibility alleles is that the genotypes ARR/VRQ and AHQ/VRQ are not very susceptible to scrapie (Detwiler and Baylis, 2003), and so even in the presence of scrapie there will be less selection against the VRQ allele when present in these genotypes.

Conversely, it may be that the VRQ allele confers an (unknown) advantage to the sheep and that it is linked to a positive trait or traits which are being selected for by farmers (Woolhouse *et al.*, 2001). Examples of this may be productivity, or other currently unknown advantages, such as increased resistance to other diseases. The possible VRQ advantage could be related to natural function of the *PrP* gene, which is currently unknown.

The *PrP* gene is present and conserved in many animals, although it is apparently not necessary for survival. Initial studies have shown that *PrP*-null mice can live

normally (in laboratory conditions) for up to two years (Büeler *et al.*, 1992; Lipp *et al.*, 1998). Removal of PrP^c does not appear to affect normal development: normal activity and avoidance behaviour have been observed in these *PrP*-null mice, as well as appropriate anxiety responses to external stimuli; and locomotion does not appear to be affected by the loss of PrP^c (Lipp *et al.*, 1998; Roesler *et al.*, 1999). However, more recent studies have indicated that a lack of PrP^c results in impaired learning ability, and impaired short-term and long-term memory (Coitinho *et al.*, 2003; Criado *et al.*, 2005). PrP^c does appear to be involved in regulating sleep cycles (Tobler *et al.*, 1996; Tobler *et al.*, 1997; Huber *et al.*, 1999); and in conjunction with copper, behaves similarly to superoxide dismutase, protecting cells from oxidative stress (Brown *et al.*, 1997; Brown *et al.*, 1999). This suggests that the effects of PrP^c are physiological rather than physical, and that any association between physical productive traits and susceptibility to scrapie are very slight so the selective advantage will be hard to detect.

Recently, there have been a number of studies assessing the impact of *PrP* genetics on productivity in sheep, most of which have not detected any valid associations between the two variables. For example, Alexander *et al.* (2005) investigated the effect of the polymorphisms at codon 171 on productivity in Suffolk sheep, and found that sheep not encoding arginine (R) at codon 171 on either allele had more lambs, with a higher overall weight at weaning, than those that encoded R on one allele; and that both of these groups of sheep had more lambs in a litter than RR_{171} sheep. This study suggests that in Suffolk sheep, there might be some association between *PrP* genotypes and lamb production. Brandsma *et al.* (2004) did also detect

some association between litter size and *PrP* genotype, and found that the genotype ARR/ARR and the VRQ allele were associated with larger litter sizes in Texel sheep. Another finding in this study was that the genotype ARR/ARR was associated with a lower 135-day weight, whereas the VRQ allele was associated with a higher 135-day weight.

However, other studies have not shown any associations with *PrP* genotype. A study on the weight of German Black-Headed Mutton sheep, suggested that sheep not encoding the ARR allele had higher weights at eight weeks of age, although this result was dismissed by the authors as it was based on a study which compared 93 sheep encoding the ARR allele to 6 not encoding this allele (de Vries *et al.*, 2004b). Another study on the lean growth rate of Suffolk sheep did not find any relationships between *PrP* genotype and growth rate (Prokopová *et al.*, 2002), and no associations between muscle mass or depth, or milk production traits, and *PrP* genotype were found in East Friesian milk sheep and German Black-Headed Mutton sheep (de Vries *et al.*, 2004, 2005). Like wise, it was determined that selection for resistant genotypes would have no effect on milk production traits in French dairy sheep breeds (Barillet *et al.*, 2002). Where some studies did detect a small association between some meat production traits and *PrP* genotype it was concluded that selection for ARR/ARR rams would not adversely affect these traits (Brandsma *et al.*, 2004; 2005).

Nevertheless, anecdotally a relationship between scrapie susceptibility and valuable production traits has long been noted among farmers, and this possible association is the focus of this thesis. Past observations have implied that is was the 'superior'

show animals, or their close relatives or offspring which were those that seemed to succumb to scrapie more often (Parry, 1962). It also has been mentioned repeatedly by farmers enrolled in the IAH field-based scrapie study (Chapter 2, page 23) that their 'worst' sheep were of resistant genotypes (pers. obs.). Steele (1964) also mentioned cases from his own records in which lambs with superior liveweight gain carried the scrapie susceptibility trait. These observations cannot be easily generalised, but do suggest that scrapie genotypes may have some importance to the fitness of sheep beyond susceptibility to the disease itself.

This association with productivity may not be due to the direct effect of the *PrP* gene, but instead be a result of the *PrP* gene influencing or linked to other nearby genes (QTL – quantitative trait loci) on the same chromosome (13 q15: Castiglioni *et al.*, 1998; Iannuzzi *et al.*, 1998). However, this is unlikely to be the case as no QTL studies have mapped 'productivity genes/loci' to the same chromosome, apart from the 'Agouti' locus, which codes for wool pigmentation (Purvis and Franklin, 2005). QTL for major productivity traits have been found on several other chromosomes, some of which are presented in table 1.2. It is unlikely that the *PrP* gene would be affecting the expression of most of these loci

Table 1.2 QTL for some productivity traits found in sheep.

Trait		Breed	Chromosome	Reference
Meat	Muscle Hypertrophy	Belgian Texel	18	a
Production	Callipye / Carwell Locus	Poll Dorset / NZ Texel	2 , 18	b
	Muscle Depth	Suffolk	1	c
	Muscle Depth; Muscle Weight; Fat Weight	Texel	18	c, d
	Muscle Weight/ 8 Week; Scan Weight	Texel	2	d
	8 Week And Scan Weight	Suffolk	18	d
Milk	Milk / Fat / Protein Content	Sarda X Lacune	OAR3, 16, 20	e
Production	Protein Percentage / Yield	Chunra	6	e
	Fat / Protein Content; Protein Yield	Lancune / Manech	5,6,9	e
	Fat Content	Lancune / Manech	9	e
Wool	Fibre Diameter	Various	1,6,25	f
Production	Fibre Strength	Various	3,7,11,25	f
	Fibre Colour	Various	13 (Agouti locus)	g

a – Laville *et al.*, 2004

b – Walling *et al.*, 2001

c – Walling *et al.*, 2004

d – Walling *et al.*, 2002

e – Barillet *et al.*, 2005

f – Purvis and Franklin, 2005

g – Parsons *et al.*, 1999

1.6 Research Aims and Objectives

The theory that scrapie-susceptible sheep are more productive had not been formally tested on a large-scale study prior to 2002. It is the focus of this study and was investigated using several approaches, which are outlined below as they are presented in this thesis.

Chapter 3: Are scrapie-susceptible sheep rated more highly by farmers?

This involves a novel approach to the investigation between *PrP* genotype and productivity which assesses farmer selection itself and its relationship to *PrP* genotype. Historically, farmer selection has been done subjectively on the basis of visual inspection and intuition. This part of the study attempts to mimic this approach by asking farmers to rate sheep on a numerical score, which can then be compared to genotype, to evaluate if susceptible sheep are indeed rated more highly by farmers.

Chapter 4: Is there an effect of genotype on culling decisions made by farmers?

As part of the ongoing IAH field-based scrapie study, many flocks have been genotyped, and this has produced a large database of the age and genotype of several thousand sheep, as well as the scrapie status of the farms involved which can be analysed to investigate possible farmer selection. As part of this IAH study, the farmers were asked to return their cull records to IAH before the genotypes are returned to the farmer: this allows quantification of actual farmer selection by genotype. The survival times of susceptible and resistant genotypes can then be compared and used to assess the impact of potential farmer selection on the genotype profile of the flock. This is similar to the study in Chapter 3 above, as it is also based on a farmer's opinion of their sheep, but instead focuses on what the farmer actually chooses to do with the sheep.

Chapter 5: Longitudinal lamb study.

The progress of lambs from a selected scrapie-free farm was followed over the course of a year. A cohort of lambs from this farm has had their growth rate assessed by recording birth weight (where possible), 8-week weight, weight at 20-21 weeks of

age, and mature weight (~11 months of age), and comparing the weights and growth rates at the different time points to genotype, while accounting for background effects, such as the environment and sire. There was also Signet information for this farm available for analysis. Chapter 5 and 6 differ to the approaches used in earlier chapters, as here scientifically measured productivity parameters are compared to genotype, as opposed to the subjective score and decisions used in Chapters 3 and 4.

Chapter 6: Can we detect a relationship between genotype and heritable traits of interest to sheep farmers?

Signet, a consultancy firm of the Meat and Livestock Commission, holds the Estimated Breeding Values (EBVs) and production indices for around 700 flocks, and claims that many of these have had their sheep genotyped. Of these flocks, nine are already part of an IAH field-based scrapie study, and have agreed to allow the use of their Signet data in a formal analysis of the genotype/productivity relationship, by comparing these Signet parameters to genotype, and other possible confounding variables such as age.

Chapter 7: Modelling how effective various breeding strategies are at reducing the risk of scrapie in individual sheep flocks.

The modelling in this chapter is based around the studies of the transmission dynamics of a flock of Romanov sheep (Hagenaars *et al.*, 2003), and aims to investigate what level of selection would be required to eliminate scrapie from an individual flock. If the selection pressure against susceptible alleles required to do

this was low, then the potential for inbreeding and the subsequent reduction in genetic gain and merit would be lowered.

2 General Materials and Methods

2.1 The IAH Field-based Scrapie Study

Between 1998 and 2004, the IAH conducted a separate farm-based scrapie field study, data from which was used in this PhD project. This study which had several aims:

1. To identify farm-level risk factors for scrapie;
2. To record the genotypes present in sheep flocks in the UK, both with and without scrapie;
3. To investigate whether the effect of genotype on the occurrence of scrapie, as found by a detailed study of a small number of animals, are supported by a large scale field study;
4. To obtain data on the demography of UK sheep flocks.

This was a case-control study, with each scrapie-affected flock in the study being matched with a scrapie-free flock of identical breed and similar numbers. Farmers were recruited by advertising in relevant publications and at agricultural shows, and were offered free genotyping in exchange for access to their sheep flocks and husbandry information (Baylis *et al.*, 2000).

2.2 Genotyping methods and genotype groups

As part of the IAH study, 66 flocks around the UK have all their breeding stock genotyped by the following methods. A 5ml blood sample was collected from each sheep into an EDTA vacutainer, and stored at -20°C. Genotyping was performed either by sequencing of the PrP gene with oligonucleotides 4142 or 9612 as

described in Baylis *et al.* (2000, 2002), or more recently with oligonucleotides 827 on a CEQ 8000 DNA capillary sequencer as described by Goldmann *et al.* (2005).

For the analyses contained in this thesis, the *PrP* genotypes were grouped in three ways that each present different ways of describing scrapie susceptibility. The grouping of the genotypes facilitates interpretation of results, as the differences between 3-5 genotype groups is examined, as opposed to the differences between up to 15 genotypes; and improves the power of the study by reducing the degrees of freedom.

The mapping of each individual *PrP* genotype to each of these groupings is presented in table 2.1. The genotype groups used are: firstly, according to risk groups as defined by the Sheep Information Group (Dawson *et al.*, 1998); secondly, by an allelic grouping based on presence or absence of the alleles ARR and VRQ; and finally, according to the estimated rate of attack (ERA, after Detwiler and Baylis, 2003). The Allelic and ERA groupings are defined as follows:

Allelic grouping	ERA grouping (based on UK sheep only)
I : ARR allele present	a : Sheep encoding ARR/ARR
II : ARR/VRQ	b : 0 – 1 reported cases per million sheep of that genotype
III : neither allele present	c : 1 – 10 reported cases per million sheep of that genotype
IV : VRQ present	d : > 10 reported cases per million sheep of that genotype

The Risk Group classification (Dawson *et al.*, 1998; table 2.1) was used in these studies as opposed to the NSP classification (DEFRA, 2005) as the NSP scheme

focuses more on the alleles carried rather than the actual relative susceptibilities of each genotype.

Table 2.1 The susceptibilities groups that each *PrP* genotype was allocated to for the study (Detwiler and Baylis, 2003; DEFRA, 2005b).

Genotype	Risk group	Allelic group	ERA group
ARR/ARR	1	I	a
ARR/AHQ	2	I	b
AHQ/AHQ	2	III	c
ARR/ARQ	3	I	b
ARR/ARH	3	I	b
ARQ/AHQ	3	III	c
AHQ/ARH	3	III	b
ARH/ARH	4	III	c
ARQ/ARH	4	III	c
ARQ/ARQ	4	III	d
ARR/VRQ	4	II	c
AHQ/VRQ	4	IV	b
ARQ/VRQ	5	IV	d
ARH/VRQ	5	IV	d
VRQ/VRQ	5	IV	d

In this thesis, sheep in Risk group *x* are also term R*x* sheep, and these terms are used interchangeably. For example, sheep in Risk group 4 may also be referred to as R4 sheep.

3 Are scrapie-susceptible sheep rated more highly by farmers?

3.1 Introduction

Every year, most farmers select sheep for culling based on several (subjective) factors, such as their performance over the year. To balance the reduction in the number of sheep, each year lambs are chosen as replacements from a large pool of lambs, and traditionally, this has been done by a combination of visual assessment and farmer intuition, and only more recently have more scientific criteria such as Signet information (see Chapter 6) and *PrP* genotype been used. This selection of replacement breeding ewes and tup rams is a major driver of genetic change in sheep. Therefore, the following study was developed as a method of assessing if farmers select replacement animals with a higher than expected level of susceptibility to scrapie, which could help explain the persistence of the alleles associated with susceptibility, namely ARQ and VRQ. In brief, farmers scored their sheep without prior knowledge of *PrP* genotype, and these scores were then compared to the genotype.

3.2 Methods

3.2.1 Study Farms and rating methods

As part of the IAH field-based scrapie study, many farmers around the UK have had their sheep blood-sampled for *PrP* genotyping. Between 2002 and 2003, nine farms were visited (table 3.1) and the farmers judged their sheep on the following characteristics: Hardiness, Wool Quality, Conformation and Body Size, by allocating a score out of three for each characteristic. The scoring was to reflect whether the

farmer was of the opinion whether the sheep was of below average (score 1); average (score 2); or above average (score 3) for each characteristic in comparison to the rest of the sheep on the farm.

Importantly, none of the farmers chosen knew the genotype of the sheep being rated, which allowed for a blind study to be performed, in which the farmers opinion of their sheep could not be biased by genotype knowledge. The ratings were done on each sheep individually, by asking the farmers to assess each sheep by visual and physical inspection (figure 3.1). The rating scores were assumed to be a guide as to the selections made by the farmers.

Figure 3.1 Rating sheep on a Welsh farm.

A total of 1128 sheep (all ewes) were assessed (table 3.1). Farm J58 was the pilot farm for the study, and all sheep whose genotypes were unknown were rated. This was found to be too fatiguing for the farmer, so on the other farms, a policy was adopted in which only a proportion of sheep was rated according to the environment and facilities available. Only 19 sheep we rated on J14, as this was all the sheep available whose genotypes were unknown to the farmer. A reduced number of sheep were rated in two Shetland flocks as they were hill sheep, and the farmers were not able to bring all of them in for rating as they were distributed over a large area. Farms N54 and N57 are factored together in the study as the same farmer managed and rated the sheep.

Table 3.1 Summary information of the farms involved in the ratings study.

Farm	Scrapie	Location	Predominant breeds	No. of sheep scored	No. of sheep with age information
D55	No	Brecon, Wales	ch, t	227	227
D66	No	Cumbria, England	sw	83	83
J14	Yes	Brecon, Wales	ch, t	19	19
J58	Yes	Brecon, Wales	ch, t	380	368
M38	No	Brecon, Wales	ch, t	108	76
N54	No	Shetlands, Scotland	sh	64	64
N57	No	Shetlands, Scotland	sh	116	116
S56	Yes	Shetlands, Scotland	sh, sh x ch	32	32
S62	No	Norfolk, England	sh	99	98

ch – Cheviot, t – Texel, sh – Shetland, sw - Swaledale

Five main breeds and crosses were represented, all of which are known to encode valine at codon 136, apart from the Suffolk breed (seven sheep only). The different breeds of sheep were grouped as follows:

- Cheviot – all Cheviot types, including region specific Cheviot, such as Brecnock Hill Cheviots;
- Texels – all Texel sheep;

- Crossbreeds – all breed crosses, for example Texel x Cheviot, Shetland x Cheviot;
- Other – the rarer breeds present on farm which were only present in limited numbers – these include Beulah Speckled Faced, Suffolk, Welsh Hill Speckled Face, and Hill Radnor;
- Shetland – Shetland sheep.

The age of the sheep was also obtained from the farmer on the day of rating or from the IAH farm records. Where comparison was possible, the age obtained from the farmers was identical to that given in the IAH database. In some cases age data were not available (table 3.1). For analysis the sheep were initially split into three age groups which reflect sheep husbandry practices:

- Age group 1 : age <3 years (lambs and sheep up to first mating);
- Age group 2 : $3 \leq$ age <5 years (peak of reproductive ability);
- Age group 3 : age ≥ 5 years (approaching end of reproductive ability, increasing risk of being culled).

This study tested whether there is any relationship between the farmer ratings collected and genotype group, while accounting for other confounding effects such as age and breed.

3.2.2 Validation of the ratings data

On farm D55 there had already been a selection for the best sheep, which were pre-allocated to 'elite' and 'non-elite' classes; these 'elite' sheep were mostly descended from twins, and were perceived to be performing better than the other sheep on farm,

in being hardier and bigger. Additionally, it had been decided that all the ewe lambs of these sheep would be kept to retain the family line on the farm. In contrast, only some of the ewe lambs of the 'non-elite' sheep would be kept for replacement breeding ewes, with the others being destined for slaughter. The ratings from this particular farm were used to judge if the questionnaire was accurately assessing the characteristics on which a farmer would base his or her selection. The median scores of each group of sheep were compared using a Kruskal-Wallis Test, and by using discriminant analysis. Discriminant analysis determines whether groups, in this case 'elite' and 'non-elite' status overall have differing rating scores, which could form a basis for predicting the status of a selected sheep. Discriminant analysis also determines the accuracy of this prediction, that is, how well the score could be used to predict the status of a selected sheep.

3.2.3 Study Repeatability

A farmer's ability to repeat the ratings was assessed on Farm D66, on which 41 sheep were rated twice within 24 hours. Repeatability, also known as the intra-class correlation coefficient (Sokal and Rohlf, 1981), is a measure of the amount of variation between the trials, and when the coefficient is high, then most of the variation is occurring within the trials, rather than between the trials.

In this analysis, repeatability for each trait was calculated using the method described by Lessels and Boag (1987). An analysis of variance was also performed, with score as the response variable and sheep, trait and trial (each time the sheep was rated) as

explanatory factors to determine which factor contributed the most to the variation in rating score. In this analysis, an interaction was also included between trait and trial.

Another method of determining the level of agreement between ratings trials was also used – the *kappa* (κ) statistic. This was calculated using SAS® version 8. This method calculates the amount of agreement between the trials which is not expected to occur by chance (Thrusfield, 1995).

3.2.4 Determining the relationship between PrP *genotype and ratings scores*

Generalised linear modelling techniques were utilised to investigate any potential relationships between rating score and genotypes (grouped into Risk, Allelic and ERA groups as defined in Chapter 2), incorporating the effects of age and breed. The rating score was the response variable and the explanatory variables used were 'elite'/'non-elite' (D55 only), age group, breed classification and genotype group.

As the farmers tended to rank the majority of the sheep in only two out of the three possible classes (figure 3.3), the classes were combined into two groups, coded 0 and 1 (table 3.2), and fitted logistic regression models to the data. Only consecutive levels were combined together. Levels were selected according to the number of sheep present in each level: those with the smallest number of sheep were combined with sheep rated as average.

Table 3.2 Combinations of the rating scores for analysis

Farm	Original score	Recombined score	Traits
D55, D66, J58, S56	1, 2 3	0 1	All
M38, N5457	1, 2 3	0 1	Hardiness, Conformation, Body Size
	1 2, 3	0 1	Wool Quality
S62	1 2, 3	0 1	Wool Quality, Conformation
	1, 2 3	0 1	Body Size

As a large number of tests examining relationships between *PrP* genotype and rating score were performed, a number of false positive results are to be expected. This arises as the selected significance automatically allows for incorrect rejection of the null hypothesis (H_0, no relationship) in 5% (for a 95% significance level) of the tests, where H_0 is true. Here a 1% significance level was used, as well as multiple comparison techniques, to ensure that the probability of a false positive result did not exceed the threshold of significance (Truszczynska, 2002).

For each trait, initially a full model of main factors (no interactions) was fitted, and then to derive the final model, all non-significant terms (at the 1% level) were deleted, apart from genotype. Additionally, if any of the age groups were shown not to be significantly different to each other, they were logically combined, that is only consecutive age groups were combined, not age groups one and three.

3.3 Results

Figure 3.2 shows the percentages of sheep in each genotype group, as classified by ERA group. All the farms have at least 10 % ARR/ARR sheep, except N5457 which has almost no sheep of this genotype present. All the farms have sheep in the most susceptible genotype group, except D66: this farm also had the highest percentage of ARR/ARR sheep. Overall, farms with Shetland sheep as the predominant breed type had the highest proportions of sheep in the highest susceptibility groups.

Figure 3.2 The percentage of sheep in each ERA group by farm (pms – per million sheep)

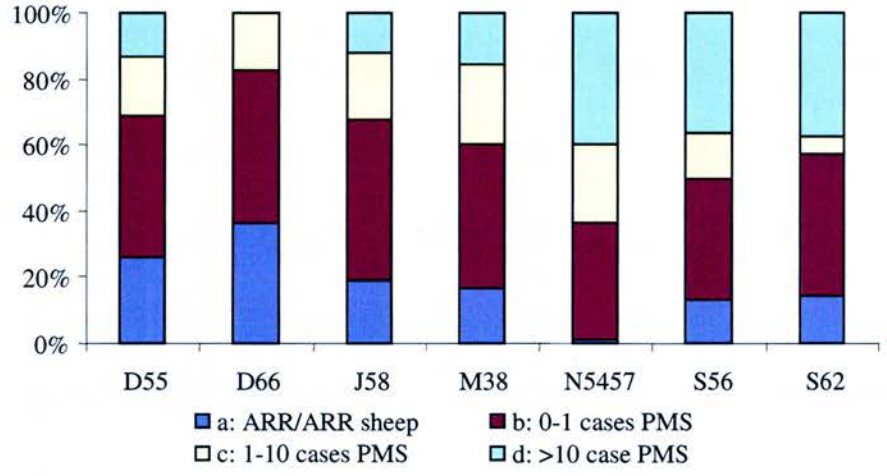
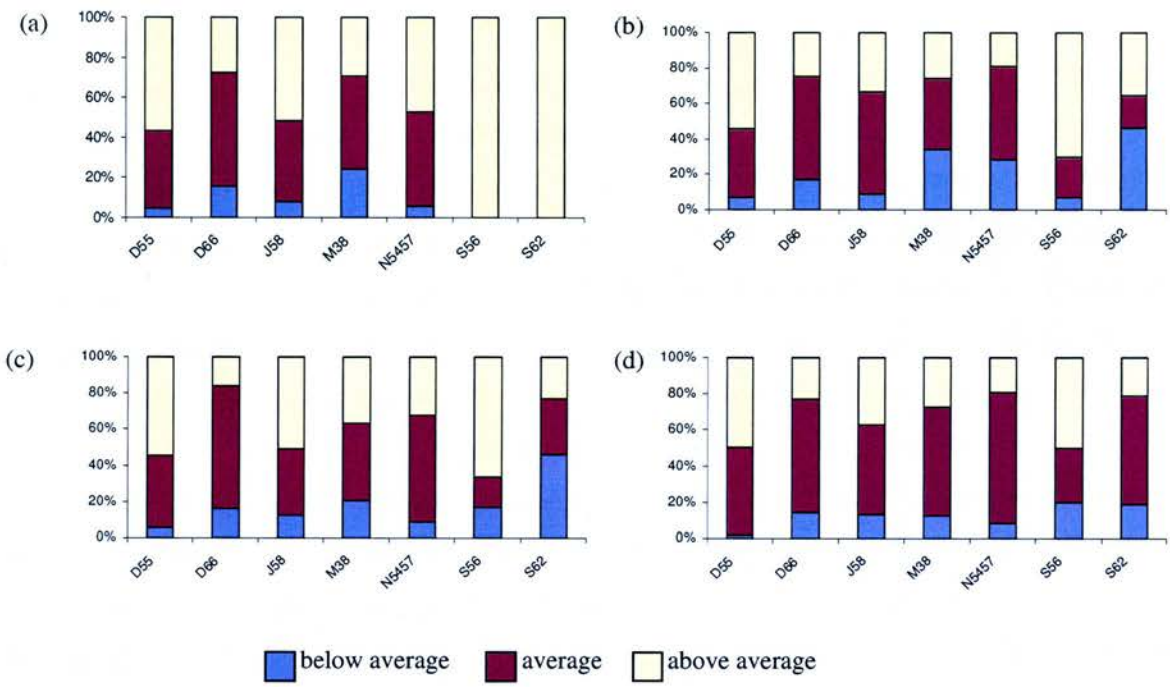


Figure 3.3 shows the percentages of sheep placed within each ratings level for the four traits Hardiness, Wool Quality, Conformation and Body Size. The majority of sheep were considered as being average or above average. Poorer sheep are under-represented, which might be due to these sheep being culled from the farm. On Farms S56 and S62 as all sheep were rated as having above average hardiness.

Information from Farm J14 is not included in the analysis, due to the very low numbers of sheep represented on this farm.

Figure 3.3 The percentages of sheep placed within each score by trait (a) Hardiness; (b) Wool Quality; (c) Conformation; (d) Body Size. The numbers of sheep represented are: D55 – 187 sheep; D66 – 69 sheep; J58 – 303 sheep; M38 – 103 sheep; N5457 – 167 sheep, S56 – 30 sheep and S62 – 91 sheep



3.3.1 Repeatability of the study

Table 3.3 presents the scores of 41 sheep rated twice by one farmer for each trait. At least 28 sheep (68%) were given the same score twice for each trait assessed. Where the scores differed, the sheep were placed one level higher or lower than its original score in all traits except Wool Quality. Here a single sheep was considered as being below average in one assessment, and above average in another.

Table 3.3 The original rating scores (1-3) of 41 sheep rated twice by one farmer for the traits Hardiness, Wool Quality, Conformation and Body Size.

Trait assessed	Trial 1	Trial 2			% rated identically
		Score 1	Score 2	Score 3	
Hardiness	Score 1	2	2	0	73
	Score 2	2	21	0	
	Score 3	0	7	7	
Wool Quality	Score 1	3	4	0	68
	Score 2	2	17	3	
	Score 3	1	3	8	
Conformation	Score 1	4	1	0	78
	Score 2	1	23	5	
	Score 3	0	2	5	
Body Size	Score 1	4	2	0	76
	Score 2	0	23	3	
	Score 3	0	5	4	

For each of the four traits, the calculated κ statistics ranged from 0.49 to 0.60 and the repeatability ranged from 0.46 to 0.61 (table 3.4) for each of the four traits. These values represent moderate agreement between the trials.

Table 3.4 The κ statistic and repeatability for each of the four traits of the 41 sheep rated twice by one farmer.

Trait	Repeatability	*kappa*	95% Confidence interval for *kappa*
Hardiness	0.54	0.53	0.30 - 0.76
Wool Quality	0.51	0.49	0.26 - 0.72
Conformation	0.61	0.60	0.36 - 0.84
Body Size	0.46	0.54	0.29 - 0.79

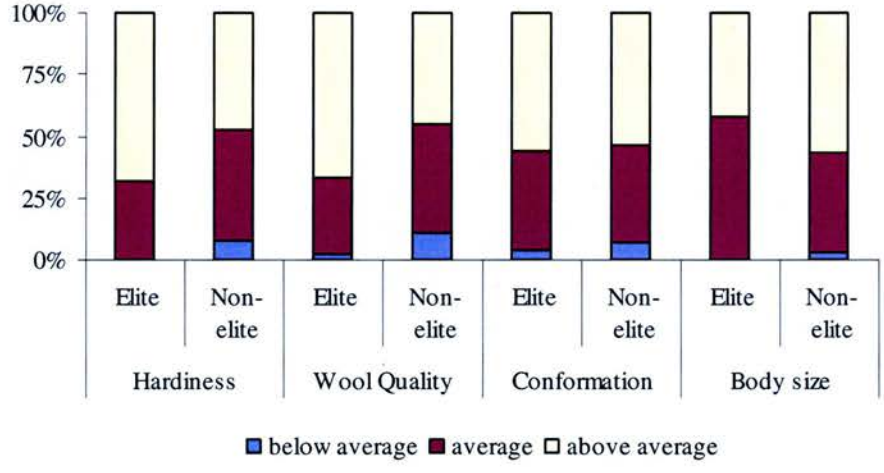
Analysis of variance with all the rating scores (irrespective of the trait they were allocated to) as the response variable and trial and individual sheep as the explanatory variables showed that there was significant variation between sheep ($p<0.001$, $df = 40$), but not between trials ($p=0.659$, $df=1$). This indicates that the rating scores between trials are similar, and thus repeatable. When this was repeated

with the scores divided by each individual trait, a similar result was obtained, that is, that most variation was between sheep, rather than between trials. Overall, it can be concluded that this method of scoring sheep is repeatable, and reliable.

3.3.2 Validation of the ratings data

The comparison of 'elite' and 'non-elite' sheep show that fewer 'elite' sheep are considered to be below average than 'non-elite' sheep in each of the four characteristics assessed. In all the traits except Body Size, more elite sheep were placed in the above average category than 'non-elite' sheep (figure 3.4).

Figure 3.4 A comparison of the percentages of 'elite' and 'non-elite' sheep within each rating level by trait



The rating scores of the 'elite' sheep were compared to those of the 'non-elite' sheep using Kruskal-Wallis tests. There were significant differences in the median rating score between 'elite' and 'non-elite' sheep for Hardiness – elite: n=84, median = 3; non-elite: n =103, median = 2, p=0.007 and Wool Quality – elite: n=84, median = 3;

non-elite: n =103, median =2, p=0.004. These p-values are two-sided, but from inspecting the median values, it can be determined that the median score for Hardiness and Wool Quality is higher in 'elite' sheep.

As there is a degree of overlap between the groups, discriminant analysis (with cross validation) was used to investigate if the ratings could be used to predict the status of the sheep, as 'elite' or 'non-elite'. The sheep were grouped with an error rate of 38.1% for 'elite' sheep, and 32.0% for 'non-elite' sheep, the overall error rate being 34.7% (table 3.5).

Table 3.5 Cross-validation table for predicting 'elite' and 'non-elite' sheep

	Classified as:		
True group	Elite	Non-elite	Error
Elite	52	32	38.1%
Non-elite	33	70	32.0%
Overall			34.7%

These results confirm that the qualities being assessed in the questionnaire are among those used by farmers to rate and select replacement breeding sheep, as pre-selected sheep appear to be regarded more highly, in at least two of the qualities assessed.

To ensure that the variations in scores were not affecting the outcomes of the analysis comparing score to susceptibility group, the models were repeated using a restricted subset of sheep with 100% repeatability, and again no significant relationships were found.

3.3.3 Determining the relationship between susceptibility and ratings scores

There were no significant ($p = 0.01$) effects of susceptibility in any of the analyses (table 3.6). Of the other factors, age, breed and 'elite' status had a significant effect on rating score, but not on all farms.

In all analyses, the results were specific to individual farms, and cannot be compared across farms. Older animals were more likely to be rated highly for hardiness (Farms 1 and 5) and conformation (Farms 1 and 7), but more likely to be rated poorly for wool quality (Farms 3 and 5). The association of age with body size was variable. The Cheviot breed were more likely to be rated as having better wool quality than other breeds of sheep on Farms J58 and M38. Texel sheep were more likely to be considered as having superior conformation than sheep in the 'Other' grouping on Farm J58; and crossbred sheep were more likely to be considered as having superior body size than Cheviot sheep on Farm D55. As seen in the Kruskal Wallace tests, there was an association between Hardiness, Wool Quality and the 'elite' sheep were more likely to be considered as having superior hardiness and wool quality to 'non-elite' sheep (Farm D55 only).

Table 3.6 A summary of the significance of the associations between the farmers' scores and genotype, age, breed and 'elite'/'non-elite' status on the score for each of the traits assessed. The full Analysis of Deviance tables are given in Appendix 1.

				Genotype Group			
Farm	Trait	Age Group	Breed	Risk Group	Allelic Group	ERA Group	Status
D55	Hardiness	0.002	ns	ns	ns	ns	<0.001
	Wool Quality	ns	ns	ns	ns	ns	0.002
	Conformation	0.005	ns	ns	ns	ns	ns
	Body Size	0.003	0.003[a]	ns	ns	ns	*
D66	Hardiness	ns	ns	ns	ns	ns	
	Wool Quality	ns	ns	ns	ns	ns	-
	Conformation	ns	ns	ns	ns	ns	
	Body Size	ns	ns	ns	ns	ns	
J58	Hardiness	ns	ns	ns	ns	ns	
	Wool Quality	**0.006**	0.002[b]	ns	ns	ns	-
	Conformation	ns	0.009[c]	ns	ns	ns	
	Body Size	**0.010**	ns	ns	ns	ns	
M38	Hardiness	ns	ns	ns	ns	ns	
	Wool Quality	ns	0.010[d]	ns	ns	ns	-
	Conformation	ns	ns	ns	ns	ns	
	Body Size	ns	ns	ns	ns	ns	
N5457	Hardiness	0.003	ns	*	ns	ns	
	Wool Quality	**<0.001**	ns	ns	ns	ns	-
	Conformation	ns	ns	ns	*	*	
	Body Size	ns	ns	ns	ns	ns	
S56	Hardiness[+]	-	-	-	-	-	
	Wool Quality	ns	ns	ns	ns	ns	-
	Conformation	ns	ns	*	ns	ns	
	Body Size	ns	ns	ns	ns	ns	
S62	Hardiness[+]	-	-	-	-	-	
	Wool Quality	ns	ns	ns	*	ns	-
	Conformation	<0.001	ns	ns	ns	ns	
	Body Size	ns	ns	ns	ns	ns	

* $0.05 < p < 0.1$

The p-values where the score allocated is lower for sheep in older age groups are in bold

[+]Farms S56 and S62 do not have results for the trait Hardiness as all sheep were rated with the same score.

[a] Crossbreed > Cheviot

[b] Cheviot > other breed present on farm

[c] Texel > 'Other'

[d] Cheviot > other breed present on farm

3.4 Discussion

The *p* values for genotype indicate that there are no relationships between scrapie susceptibility and any of the rating scores. It was necessary to assess the validity of this study, and this was achieved in two ways. Repeatability was assessed by a farmer rating the same sheep twice. For all four traits, more than two-thirds of the sheep were given the same score twice, and only in one trait was a single sheep given opposite scores over the two trials. The difference may be partly due to small changes in, for example, light conditions; but is more likely a result of the subjective nature of the rating process. However, this variation in scores is unlikely to have affected the analysis of susceptibility groups and rating score on any of the farms, as when the analysis was repeated using a restricted dataset of sheep from Farm D66 with 100% repeatability in the rating scores, again no significant relationships were found.

Secondly, at least two of the qualities assessed are among those used by farmers to select replacement sheep as, on Farm D55, 'elite' sheep were rated more highly in two of the traits, Hardiness and Wool Quality. An association between 'elite' status and Hardiness is expected and confirmed in this analysis, as sheep were selected for their perceived superior hardiness and size (although an association with Body Size was not present). A limitation of this analysis is that the farmer had clearly marked the 'elite' sheep for easy identification and the rating scores could have been biased to reflect the earlier selection. However, as many non-elite sheep were rated more highly than elites, it is unlikely that the farmer was being heavily influenced by the markings dividing the two groups of sheep.

On a number of farms, factors other than scrapie susceptibility had the most effect on ratings score, such as age and breed, despite having asked farmers to judge sheep according to their performance for the breed and age on that particular farm. Overall, older sheep were more likely to be rated as hardier, and having better conformation, although with poorer wool. The age effect on body size was more variable across the farms. Poorer wool quality in older animals is likely to reflect fleece deterioration over time. Similarly, older sheep may have better conformation, as these sheep are more mature and fully-grown than their younger counterparts. The breed effects present were variable, and can be explained mostly in the development of certain breeds for certain conditions. For example, Texels are a meat breed, and so are bred for superior conformation, rather than their durability at pasture, unlike certain other breeds, such as the Hill Radnors and Welsh Hill sheep. In the sheep industry, 'meat' breeds are commonly mated to hill breeds to produce larger ewes, which in turn are tupped by terminal sire rams to produce hardier lambs with a higher muscle to bone ratio, hence the finding on Farm D55 that crossbred sheep are more likely to have better size is not exceptional. These associations with age and breed also validate the study data; they would not be present if the farmers' score were just random and not a reflection of the performance of the sheep.

In summary, farmers' assessments of their best performing animals are not biased towards susceptible or resistant genotypes based on the characteristics judged in this study. There are no relationships between the qualities assessed and genotype, and the farmers involved are not predicting *PrP* genotype based on the productivity traits

assessed. Therefore, it is unlikely that farmer selection of replacement breeding ewes is a factor behind the persistence of scrapie-susceptible sheep.

4 Is there an effect of genotype on culling decisions made by farmers?

4.1 Introduction

The purpose of this part of the study was to assess whether there is any bias towards certain *PrP* genotypes in culling decisions made by farmers. This was achieved by monitoring what had happened to the sheep after they had been blood sampled for genotyping, looking for associations between being retained on the farm and *PrP* genotype.

The study is divided into three sections with all sections allowing a comparison of the performance and survivorship of susceptible sheep on scrapie-affected and scrapie-free farms. The first section is similar to that in Chapter 3, as it also focuses on a farmer's perception of their sheep, rather than actual measurements of sheep performance. However, in this part of the study, whether farmers retain their sheep on farm or not is examined rather than farmers assessing each sheep and allocating it a score based on certain physical characteristics. The choice to keep a sheep on farm is taken as an indicator of higher performance, with the assumption that less-fit or worse-performing sheep are more likely to be selected by management for culling. The status 'culled' or 'not culled' can be compared to *PrP* genotype to assess whether sheep susceptible to scrapie are less likely to be chosen for culling.

The second section of the study evaluates the survivorship of sheep of different levels of susceptibility to scrapie. This section is more of a follow-up study which examines the survival of sheep on scrapie-free and scrapie-affected farms after the genotypes are revealed to the farmer, that is what farmers actually have chosen to do with their sheep once the *PrP* genotypes are known: it is expected that there would be selection against susceptible genotypes. Losses due to scrapie are excluded from this study, with all other causes for removal from a flock being included, whether from culling or from natural causes.

The third section of this study is concerned with those sheep reported as being found dead from both known and unknown causes on the farms. The proportions of these sheep were compared, and associations between genotype, age, and farm scrapie status were investigated. This was undertaken as previous studies have shown that more sheep die of unknown causes on scrapie-affected farms (McLean *et al.*, 1999; Humphry *et al.*, 2004), which may be attributed to scrapie undiagnosed prior to death (Pattison *et al.*, 1974; Humphry *et al.*, 2004; Chase-Topping *et al.*, 2005).

4.2 Materials and Methods

4.2.1 Data sources

Before any subsequent visits were made, farmers taking part in the IAH field-based scrapie study (as described in Chapter 2) were requested to fill out a questionnaire indicating what had happened to the sheep that were blood sampled on the first visit, but were no longer on the farm. These data were then divided for the three parts of the study:

1. Information on sheep which were culled before the farmer knew their genotype. This part of the study is referred to as the Blind Cull study.
2. Information on sheep which were removed from the farm, but did not die of scrapie, regardless of cause. This part of the study is referred to as the Survivorship study.
3. Information on the numbers of sheep found dead of known and unknown causes on scrapie-affected and scrapie-free farms. This is referred to as the Found Dead study.

In all three sections of the study, a 5% significance level was used.

Sheep are culled as part of flock management, and replaced by lambs. The association between removal status and *PrP* genotype was examined to see if there is farmer selection for susceptible genotypes, which might explain the persistence of susceptible alleles in the face of negative selection from scrapie.

The IAH farm database, which contains the details of all the farms involved in the IAH field-based study, was examined retrospectively and farms were extracted which had 'fates information': the details for all the sheep which were no longer recorded as being on the farm, whether they had died (from scrapie, other diseases or of unknown causes) or were sold.

The information extracted for this study represents 3384 sheep, which were sampled over the years 1998, 1999, 2000 and 2002. There was information available on 10 farms for the Blind Cull study, six scrapie-affected and four scrapie-free. For the

Survivorship Study, there were data available for 14 farms, six of which were scrapie-free. These are summarised in table 4.1.

Table 4.1 Summary information of the farms extracted from the IAH database for this study, and which sections the data was used in.

Farm	Scrapie Present	Year of Blood Sampling	Number of Sheep Genotyped	Study Section
A35	N	2000	123	1,2, 3
C16	Y	1998	190	2, 3
D12	Y	1998	239	1, 2, 3
D34	N	2000	359	1, 2, 3
H19	N	1999	303	2, 3
H37	N	2000	375	1, 2, 3
J09	Y	1998	183	2, 3
J58	Y	2002	255	1
M28	Y	1999	85	1, 2, 3
M30	N	1999	104	2, 3
P27	Y	1999	123	1, 2, 3
S03	Y	1998	135	2, 3
T36	Y	2000	455	1, 2, 3
T59	Y	2002	330	1, 2, 3
U29	N	1999	125	1, 2, 3

For each of the extracted farms, the following information was noted: the scrapie status of the farm; the total number of sheep sampled on the first visit; their years of birth (YoB); their genotypes; which sheep were lost from the flock; and the length of time after they were blood sampled that the sheep were lost from the farm. Only females were included in the datasets, as there was only limited information available on male sheep.

4.2.2 Analytical methods

Section 1 – Blind Cull Study

Only a limited number of sheep from each farm were culled before their genotypes were known, so the farms were grouped and generalised linear mixed models

(Genstat version 8®) were used for analysis. A binomial error structure was used, with the response variables being 'culled' and 'not culled'. Survival analysis was not used as the sheep were removed on one or two dates only, when the farmer had selected out sheep for management culling.

The explanatory variables were YoB, genotype group (as defined in Chapter 2: Risk group, Allelic group and ERA group), Farm and Scrapie status. The analysis proceeded with the data grouped in two ways. Firstly, all the data were pooled, with Scrapie status, YoB and genotype group included as fixed effects and Farm as a random effect. The interaction between genotype group and Scrapie status was also investigated. Secondly, the data were split according to the scrapie status of the farm, with the explanatory variables YoB and genotype group designated as fixed effects and, once again, Farm as a random effect. This allowed for another comparison of the performance of sheep of similar genotypes on scrapie-free and scrapie-affected farms. In both cases, sheep which had died (not selected for culling) were excluded from the data set.

Section 2 – Survivorship Study

Each farm was analysed separately in this study. Cox proportional hazard models were used, with the response being the number of days that the sheep remained on the farm after sampling. The observation period was taken as the number of days between the sampling date and the last known date that the sheep were known to be present on the farm. All sheep recorded as 'alive' at the end of this study were right censored, and all other animals were classified as 'dead' or 'removed' including

those selected for culling by the farmer, as well as natural losses due to disease and idiopathic deaths. Sheep which died of scrapie were excluded from this study, to allow investigation of the survivorship and performance of susceptible sheep on scrapie-affected farms in the absence of clinical disease. Here, YoB and genotype group were included as explanatory variables.

Section 3 – Found Dead Study

This study involved sheep found dead on the farm of known and unknown causes, but excluded known scrapie deaths. Chi-squared and Fisher Exact Tests were used to investigate possible associations between a sheep being found dead and the scrapie status of the farm, genotype group or YoB. A series of univariate comparisons were made, which compared the numbers of sheep found dead of known and unknown causes to:

- the scrapie status of the farm,
- the YoB of the sheep,
- the genotype (group) of the sheep.

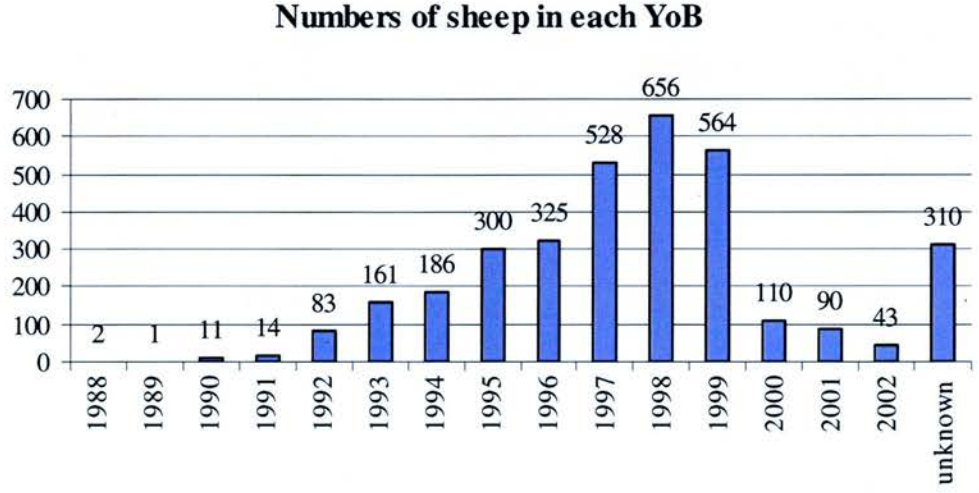
The numbers of sheep found dead of each genotype were also compared on scrapie-free and scrapie-affected farms, and possible interactions between the scrapie status and the genotype of the sheep were also investigated, by comparing the numbers of sheep of each genotype found dead of known and unknown causes on scrapie-affected and scrapie-free farms.

4.3 Results

4.3.1 Data sources

The information from the IAH field-based scrapie study represents 3384 sheep, with information on 10 farms for the Blind Cull study, six scrapie-affected and four scrapie-free; and 14 for the Survivorship Study, six of which were scrapie-free. The numbers of sheep represented on each of the farms, as well as the numbers included in each section of the study are presented in the relevant sections below. Just over half of the sheep (1748 / 3384) involved in the study were born in the years 1997 – 1999. There were very few sheep present that were born prior to 1993, and likewise few born after 1999 in the study (figure 4.1) so for the analyses, YoB cohorts were combined initially to form seven age groups: ≤ 1993, 1994, 1995, 1996, 1997, 1998 and ≥ 1999.

Figure 4.1 The YoB distribution for the 3384 sheep represented in this study.



The genotype distributions by the grouped YoB are shown in figures 4.2a – c. From the graphs, it is apparent that there is a very low prevalence of ARR/ARR sheep, with this genotype representing around 10 – 20% of sheep in each of the YoB cohorts (figure 4.2a, c); and the ARR allele is present in 30 – 70% of sheep in each of the years represented (figure 4.2b). There is also a higher percentage of sheep in ERA group d sheep than group a in each of the YoB represented (figure 4.2c).

Figure 4.2 The percentage of sheep in genotype group by YoB: (a) Risk group; (b) Allelic Group; (c) ERA group

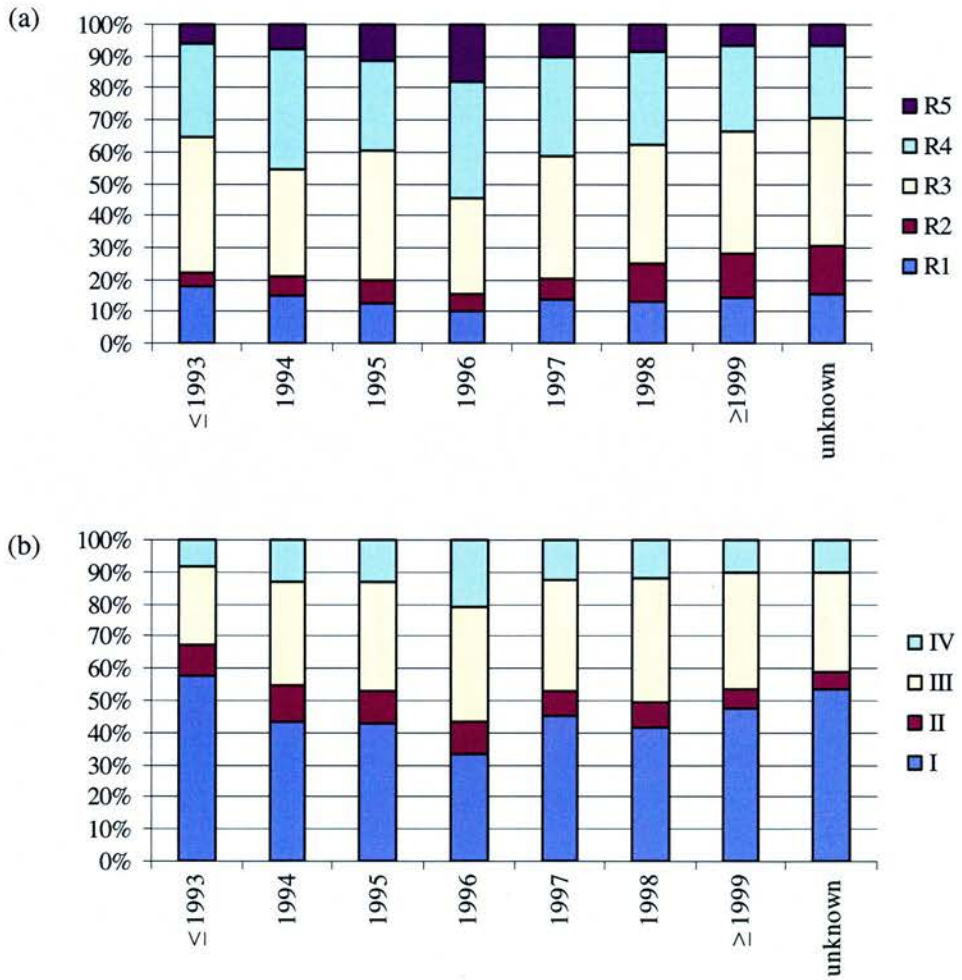
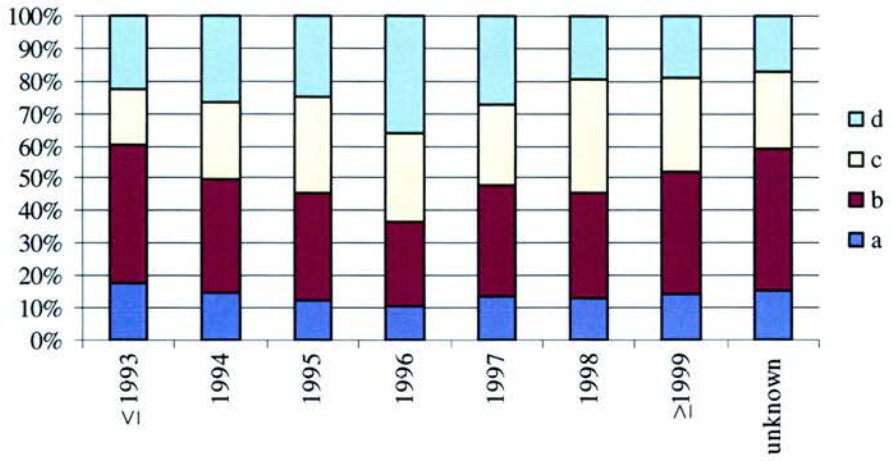


Figure 4.2c



4.3.2 Blind Cull Study

The farms which had data suitable for this study and the numbers of sheep represented on each farm are given in table 4.2. This table also indicates the numbers of sheep which died and were excluded from the analysis. Between 0.8% and 44.7% of the original number of sheep sampled were selected for culling by each of the farms with an average culling percentage of 6.5%.

Table 4.2 Summary of the farms involved in the Blind Cull study.

Farm	Scrapie present	Number of sheep genotyped	Number culled	Percentage of original number culled	Number of sheep excluded	Number used in analysis
A35	N	123	19	15.4	3	120
D12	Y	239	2	0.8	7	232
D34	N	359	16	4.5	8	351
H37	N	375	13	3.5	2	373
J58	Y	255	7	2.7	0	255
M28	Y	85	38	44.7	2	83
P27	Y	123	15	12.2	2	121
T36	Y	455	7	1.5	35	420
T59	Y	330	11	3.3	5	325
U29	N	125	32	25.6	3	122

Tables 4.3a – c present the above data grouped by genotype, that is, the original number of sheep sampled by genotype, and the proportions of each genotype culled by farm. Despite the variation between the farms, overall as the risk group number and ERA increases, the number of sheep being culled increases. This suggests that farmers are selecting against these susceptible genotypes, and under the Allelic grouping, sheep encoding ARR/VRQ were most frequently culled. However, no significant associations between YoB, genotype group or scrapie status and whether or not a sheep was selected for culling were present, whether the farms were analysed as one group, or divided by scrapie status.

Table 4.3 The number of sheep of each genotype group originally sampled and the numbers and proportions culled from each farm. The last row shows the overall number and percentage of sheep from each genotype group which were culled. a) Risk group; b) Allelic group; c) ERA group

a)

		R1			R2			R3			R4			R5		
Farm	Scrapie	total	culled	%	total	culled	%	total	culled	%	total	culled	%	total	culled	%
A35	N	19	2	10.5	8	2	25	52	6	11.5	33	8	24.2	11	1	9.1
D12	Y	48	0	0	31	0	0	93	1	1.1	59	0	0	8	1	12.5
D34	N	130	3	2.3	-	-	-	97	4	4.1	100	5	5	32	4	12.5
H37	N	2	0	0	47	3	6.4	142	4	2.8	113	3	2.7	71	3	4.2
J58	Y	49	2	4.1	48	2	4.2	97	3	3.1	53	0	0	8	0	0
M28	Y	7	0	0	-	-	-	38	17	44.7	35	18	51.4	5	3	60
P27	Y	6	3	50	-	-	-	51	8	15.7	66	4	6.1	-	-	-
T36	Y	2	0	0	112	2	1.8	180	4	2.2	121	1	0.8	40	0	0
T59	Y	55	1	1.8	79	0	0	116	5	4.3	60	2	3.3	20	3	15
U29	N	5	1	20	1	0	0	65	15	23.1	51	15	29.4	3	1	33.3
Overall		323	12	**3.7**	326	9	**2.8**	931	67	**7.2**	691	56	**8.1**	198	18	**8.1**

Table 4.3b

		I			II			III			IV		
Farm	Scrapie	total	culled	%	total	culled	%	total	culled	%	total	culled	%
A35	N	71	8	11.3	5	2	40.0	36	8	22.2	11	1	9.1
D12	Y	144	0	0	17	0	0	63	1	1.6	15	1	6.7
D34	N	227	7	3.1	88	5	5.7	12	0	0	32	4	12.5
H37	N	53	4	7.5	19	2	10.5	190	3	1.6	113	4	3.5
J58	Y	155	4	2.6	13	0	0	72	3	4.2	15	0	0
M28	Y	45	17	37.8	5	2	40	30	16	53.3	5	3	60
P27	Y	57	11	19.3	-	-	-	66	4	6.1	-	-	-
T36	Y	39	0	0	3	0	0	330	6	1.8	83	1	1.2
T59	Y	198	6	3.0	28	2	7.1	74	0	0	30	3	10
U29	N	65	15	23.1	-	-	-	57	16	28.1	3	1	33.3
Overall		1054	72	**6.8**	178	13	**7.3**	930	57	**6.1**	307	18	**5.9**

Table 4.3c

		a			b			c			d		
Farm	Scrapie	total	culled	%	total	culled	%	total	culled	%	total	culled	%
A35	N	19	2	10.5	52	6	11.5	13	4	30.8	39	7	17.9
D12	Y	48	0	0	105	0	0	47	1	2.1	39	1	2.6
D34	N	130	3	2.3	97	4	4.1	89	5	5.6	43	4	9.3
H37	N	2	0	0	93	5	5.4	166	5	3.0	114	3	2.6
J58	Y	49	2	4.1	122	2	1.6	54	3	5.6	30	0	0
M28	Y	7	0	0	38	17	44.7	5	2	40	35	19	54.3
P27	Y	6	3	50	51	8	15.7	62	4	6.5	4	0	0
T36	Y	2	0	0	87	1	1.1	257	6	2.3	109	0	0
T59	Y	55	1	1.8	153	5	3.3	80	2	2.5	42	3	7.1
U29	N	5	1	20	66	15	22.7	50	14	28	4	2	50
Overall		323	12	**3.7**	864	63	**7.3**	823	46	**5.6**	459	39	**8.5**

The analysis was then repeated using just the farms with a culling percentage above 10% (A35, M28, P27 and U29). With the four farms grouped together, no associations were present between scrapie status or genotype and being culled, although the risk of being culled increased with age ($p = 0.012$). When the farms were divided by scrapie status, no association between any of the explanatory factors and being culled were present on the scrapie-affected farms, whereas the risk of being culled increased with age on the scrapie-free farms ($p = 0.009$). Again genotype did not affect the risk of being culled on the scrapie-free farm.

Each of these four farms with the highest cull rate were then analysed individually. On the two scrapie-free farms (A35 and U29), there was only an association with YoB, with younger sheep (up to two years of age), being preferentially selected for culling ($p = 0.005$ and $p = 0.002$, respectively). On one scrapie-affected farm, M28, there was no association between any of the explanatory variables and being culled. On P27, however, there was an association with genotype. Although the model including risk group was significant ($p = 0.017$), the risks of being culled were not significantly different to the reference level (Risk group 3, table 4.4). No sheep in ERA group d were culled, so the groups c and d were combined. Sheep in this combined group were very unlikely to be culled (table 4.4), compared to sheep in ERA group a ($p = 0.017$).

Table 4.4 Odds ratios of sheep being culled in genotype groupings Risk group (reference group 3) and ERA group (reference group a) on Farm P27. LCL – Lower 95 % Confidence Limit, UCL – Upper 95% Confidence Limit.

Genotype Group	Odds Ratio	LCL	UCL	p-value
Risk Group 1	5.38	0.92	31.54	0.062
Risk Group 4	0.36	0.10	1.27	0.111
ERA Group b	0.19	0.03	1.09	0.062
ERA Groups c and d	0.07	0.01	0.44	0.005

4.3.3 Survivorship Study

The farms involved in this study are presented in table 4.5. This table also includes the observation period observed for each farm, as well as the numbers of sheep which were excluded from the study as they had died from scrapie.

Table 4.5 Summary of the farms involved in the Survivorship study, and the observation periods for each farm.

Farm	Scrapie present	Number of sheep genotyped	Number lost	Percentage of original number lost	Observation time (dps*)	Number of scrapie deaths	Number used in analysis
A35	N	123	33	26.8	265	n/a	123
C16	Y	190	154	81.1	1105	3	187
D12	Y	239	34	14.2	701	1	238
D34	N	359	89	24.8	348	n/a	359
H19	N	303	129	42.6	770	n/a	303
H37	N	375	16	4.3	359	n/a	375
J09	Y	183	92	50.3	788	10	173
M28	Y	85	52	61.2	663	0	85
M30	N	104	76	73.1	1036	n/a	104
P27	Y	123	63	51.2	759	0	123
S03	Y	135	77	57.0	643	0	135
T36	Y	455	85	18.7	685	6	449
T59	Y	330	16	4.8	190	0	330
U29	N	125	48	38.4	414	n/a	125

* dps – days post sampling
n/a – not applicable

As the observation periods across the farms were highly variable, each farm was analysed separately. Across all the farms, the overall percentage of sheep lost from those originally sampled was 30.8%, with a range of 4.3% to 81.1%. Tables 4.6a – c present the above data for the Survivorship Study, grouped by genotype, with the figures shown reflecting the proportions of sheep of each genotype lost from the number originally sampled. Overall, as the Risk group number and ERA increases, the proportion of sheep removed from the flock increases suggesting that there may be selection against susceptible genotypes. Under the Allelic grouping, sheep encoding the VRQ allele (in groups II and IV, 33.3% and 36.3% respectively) were most frequently lost from the flocks, with specifically ARR/VRQ sheep (group II) being the most frequently removed.

Table 4.6 The number of sheep of each genotype group originally sampled and the numbers and proportions lost the flock on each farm. The last row shows the overall number and percentage of sheep from each genotype group which were lost. (a) Risk group; (b) Allelic group; (c) ERA group

(a)

		R1			R2			R3			R4			R5		
Farm	Scrapie	Original number	lost	%	Original number	Lost	%	Original number	Lost	%	Original number	Lost	%	Original number	Lost	%
A35	N	19	3	15.8	8	2	25	52	12	23.1	33	13	39.4	11	3	27.3
C16	Y	38	30	78.9	9	6	66.7	81	63	77.8	52	46	88.5	10	9	90
D12	Y	48	2	4.2	31	0	0	93	6	6.5	59	18	30.5	8	8	100
D34	N	130	24	18.5	-	-	-	97	23	23.7	100	33	33	32	9	28.1
H19	N	66	29	43.9	-	-	-	102	52	51	100	41	41	35	7	20
H37	N	2	0	0	47	3	6.4	142	6	4.2	113	4	3.5	71	3	4.2
J09	Y	18	5	27.8	-	-	-	55	27	49.1	72	27	37.5	38	33	86.8
M28	Y	7	0	0	-	-	-	38	23	60.5	35	24	68.6	5	5	100
M30	N	5	2	40	-	-	-	44	27	61.4	39	31	79.5	16	16	100
P27	Y	6	4	66.7	-	-	-	51	24	47.1	66	35	53	-	-	-
S03	Y	18	10	55.6	-	-	-	61	38	62.3	46	23	50	10	6	60
T36	Y	2	0	0	112	19	17	180	33	18.3	121	22	18.2	40	11	27.5
T59	Y	55	2	3.6	79	1	1.3	116	6	5.2	60	3	5	20	4	20
U29	N	5	1	20	1	0	0	65	21	32.3	51	25	49	3	1	33.3
Total		419	112	**26.7**	287	31	**10.8**	1177	361	**30.7**	947	345	**36.4**	299	115	**38.5**

Table 4.6b

		I			II			III			IV		
Farm	Scrapie	Original number	lost	%	Original number	Lost	%	Original number	Lost	%	Original number	Lost	%
A35	N	71	14	19.7	5	3	60	36	13	36.1	11	3	27.3
C16	Y	110	85	77.3	20	19	95	49	40	81.6	11	10	90.9
D12	Y	144	6	4.2	17	8	47.1	63	8	12.7	15	12	80
D34	N	227	47	20.7	88	28	31.8	12	5	41.7	32	9	28.1
H19	N	168	81	48.2	29	13	44.8	71	28	39.4	35	7	20
H37	N	53	4	7.5	19	2	10.5	190	6	3.2	113	4	3.5
J09	Y	73	32	43.8	32	7	21.9	40	20	50	38	33	86.8
M28	Y	45	23	51.1	5	4	80	30	20	66.7	5	5	100
M30	N	48	28	58.3	7	5	71.4	33	27	81.8	16	16	100
P27	Y	57	28	49.1	-	-	-	66	35	53	-	-	-
S03	Y	79	48	60.8	6	2	33.3	40	21	52.5	10	6	60
T36	Y	39	9	23.1	3	0	0	330	52	15.8	83	24	28.9
T59	Y	198	9	4.5	28	3	10.7	74	0	0	30	4	13.3
U29	N	65	21	32.3	-	-	-	57	26	45.6	3	1	33.3
Total		1377	435	**31.6**	259	94	**36.3**	1091	301	**27.6**	402	134	**33.3**

Table 4.6c

		a			b			c			d		
Farm	Scrapie	Original number	lost	%	Original number	Lost	%	Original number	Lost	%	Original number	Lost	%
A35	N	19	3	15.8	52	11	21.2	13	6	46.2	39	13	33.3
C16	Y	38	30	78.9	74	57	77	40	33	82.5	38	34	89.5
D12	Y	48	2	4.2	105	8	7.6	47	10	21.3	39	14	35.9
D34	N	130	24	18.5	97	23	23.7	89	28	31.5	43	14	32.6
H19	N	66	29	43.9	102	52	51	29	13	44.8	106	35	33
H37	N	2	0	0	93	5	5.4	166	8	4.8	114	3	2.6
J09	Y	18	5	27.8	55	27	49.1	32	7	21.9	78	53	67.9
M28	Y	7	0	0	38	23	60.5	5	4	80	35	25	71.4
M30	N	5	2	40	43	26	60.5	8	6	75	48	42	87.5
P27	Y	6	4	66.7	51	24	47.1	62	31	50	4	4	100
S03	Y	18	10	55.6	61	38	62.3	6	2	33.3	50	27	54
T36	Y	2	0	0	87	23	26.4	257	42	16.3	109	20	18.3
T59	Y	55	2	3.6	153	7	4.6	80	3	3.8	42	4	9.5
U29	N	5	1	20	66	21	31.8	50	24	48	4	2	50
Total		419	112	**26.7**	1077	345	**32.0**	884	217	**24.5**	749	290	**38.7**

After model simplification, in which non-significant terms (at the 5% level) were eliminated from the model, there were no relationships between YoB or genotype group and survival on Farm H37. On five farms, A35, C16, H19, S03 and T36, survival was associated with YoB only ($p < 0.001$) and on two farms, J09 and U29, survival was associated with YoB and genotype ($p < 0.001$). On six farms, D12, D34, M28, M30, P27 and T59, there were associations with genotype only.

On six out of seven farms for which YoB had a significant effect (A35, C16, H19, J09, S03 and T36) increasing age of the sheep was associated with reduced survival (figure 4.3a), whereas on farm U29, sheep born after 1996 appear more likely to be lost from the flock than those born prior to 1996 (figure 4.3b also see Appendix 2, page 212).

Figure 4.3 Kaplan-Meier curves highlighting the association between YoB and survival. (a) a typical result showing decreased survival with increasing age (decreasing YoB, Farm C16). (b) the result for Farm U29

(a)

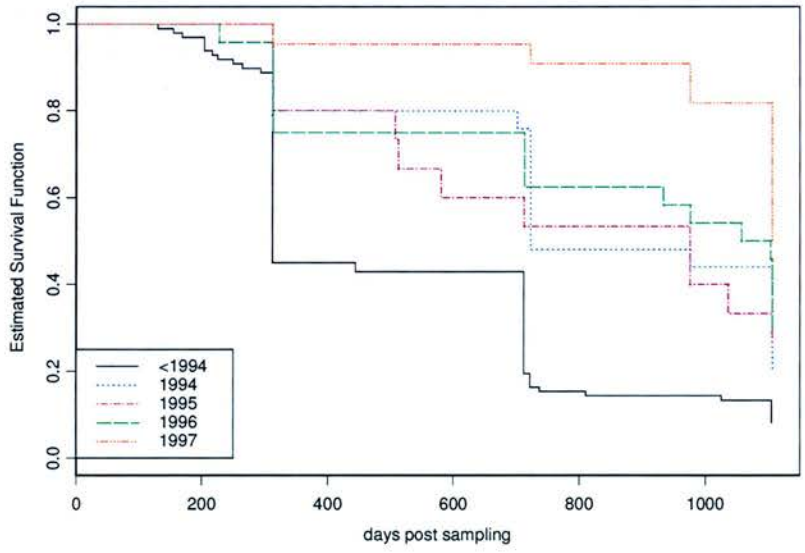
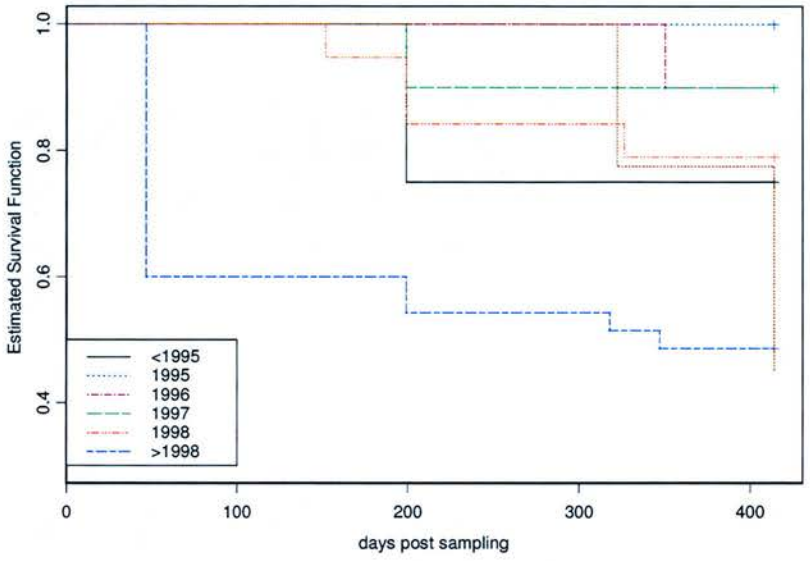


Figure 4.3b



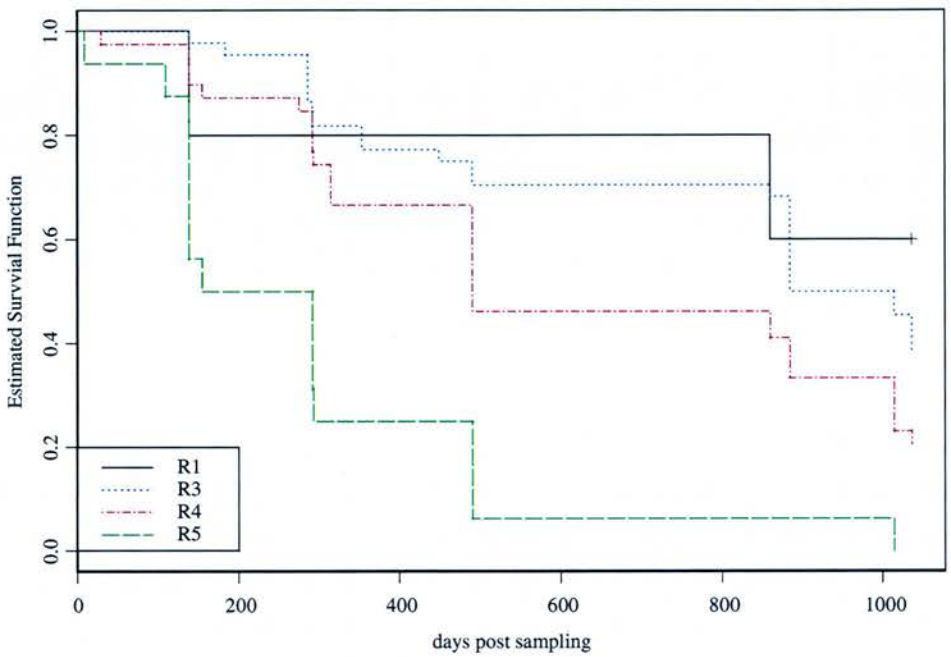
On four of the eight farms on which there is an association between genotype and survival (D34, P27, T59 and U29), genotype was not significant in terms of model fit, but included a hazard ratio which was significantly different from one (table 4.7, highlighted in bold, Appendix 2). In all analyses, on both scrapie-affected and scrapie-free farms, sheep highly susceptible to scrapie have lower survival than more resistant sheep (figure 4.4)

Table 4.7 Farms on which there are associations between survival and genotype.

	Farm							
Genotype group	D12	D34	J09	M28	M30	P27	T59	U29
Risk Group	<0.001	-	0.005	0.024	<0.001	-	0.061	-
Allelic	<0.001	0.126	0.001	0.048	<0.001	-	-	-
ERA	<0.001	0.094	0.001	-	0.004	0.103	-	0.306

Figure 4.4 Kaplan-Meier curves highlighting the association between genotype group and survival (Farm M30). (a) Risk group; (b) Allelic group; (c) ERA group

(a)



(b)

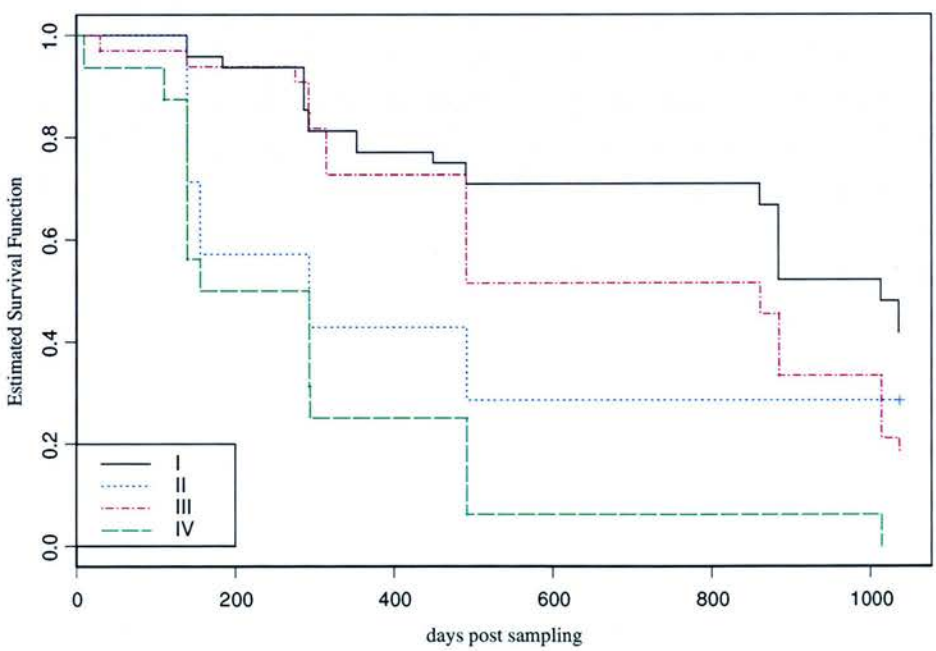
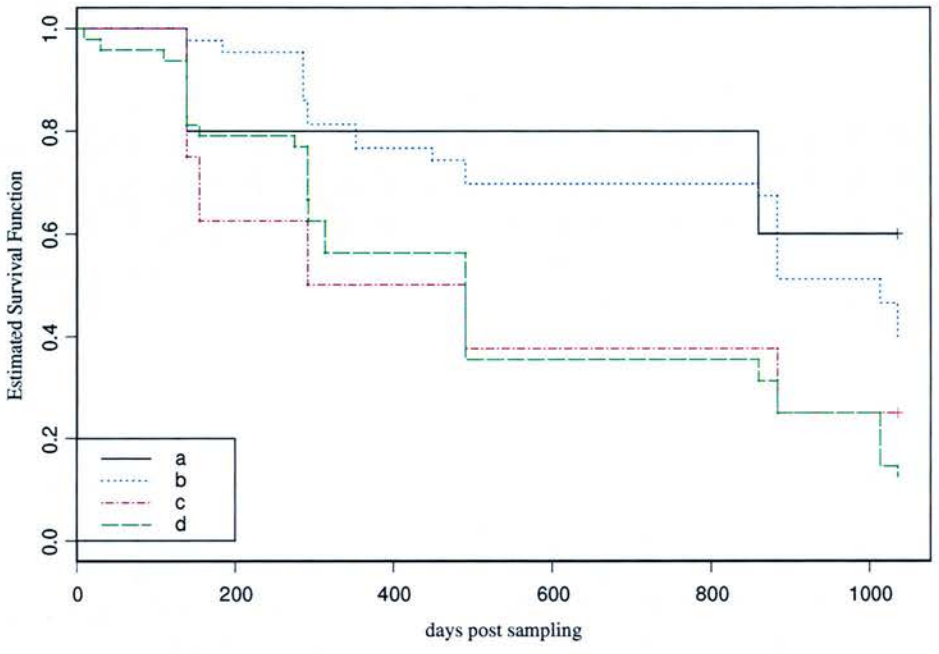


Figure 4.4c



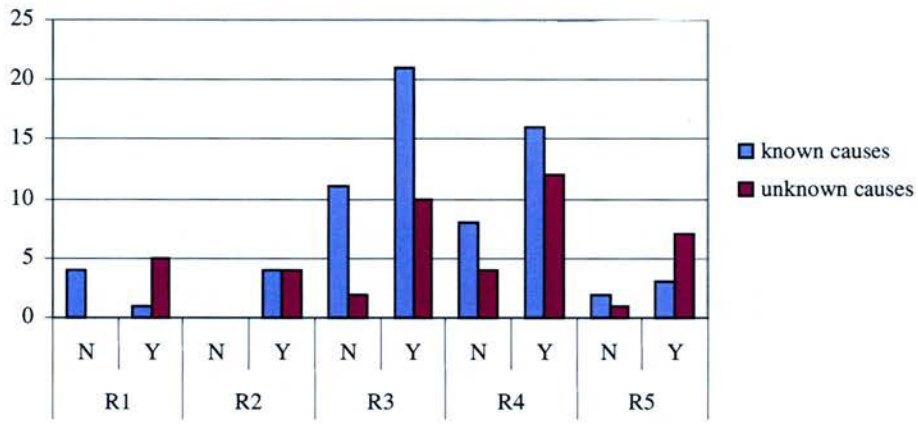
4.3.4 Found Dead Study

On scrapie affected-farms, more sheep died of unknown causes than on scrapie free farms (χ^2 =5.54, df = 1, p=0.019). However, there was no association between YoB (Fishers Exact Test – FET, p = 0.145) or genotype (FET: Risk group, p = 0.171; Allelic group, p = 0.229; ERA group, p = 0.467) and being found dead of unknown causes.

To test for interaction effects between scrapie status and genotype, a multilevel analysis was performed (using a 3-dimensional table). There was an interaction between genotype and the scrapie status of the farms in relation to the number of sheep found dead (FET, p = 0.04, figure 4.5). On the scrapie-affected farms, more R1 and R5 sheep died of unknown causes than known causes. The reverse is true for

sheep in Risk groups 2 – 4, with equivalent numbers or more dying of known causes for these genotypes. On the scrapie-free farms, more sheep died from known causes than unknown causes across all the genotypes (figure 4.5).

Figure 4.5 The numbers of sheep of each Risk group found dead of known and unknown causes on scrapie affected (Y) and scrapie unaffected (N) farms.

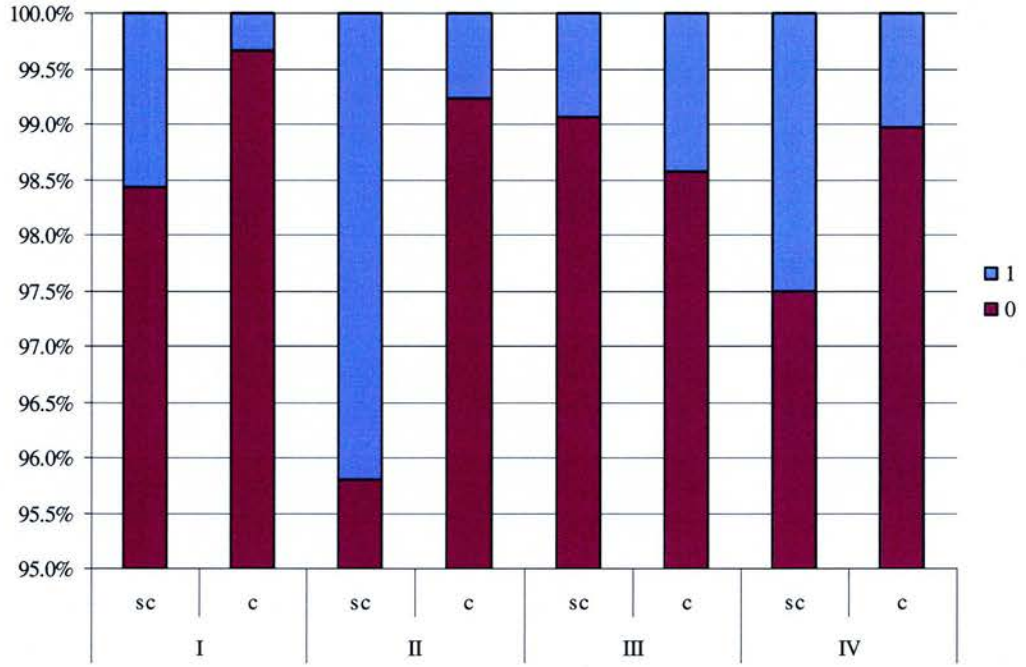


However, when considering only the sheep that died of unknown causes, there was no effect of genotype on being found dead (of unknown cause) on either scrapie-free or scrapie-affected farms (FET: Risk group, $p = 0.798$; Allelic group, $p = 0.843$; ERA group, $p = 0.520$).

Finally, the proportions of sheep of each genotype found dead of unknown causes on scrapie-affected farms was compared. A higher proportion sheep encoding the VRQ allele (Allelic groups II and IV) were found dead on scrapie farms (figure 4.6, $\chi^2 = 10.36$, $df = 3$, $p = 0.016$) compared to other genotypes represented, whereas there was no genotype bias on scrapie-free farms (Risk group: $\chi^2 = 3.30$, $df = 4$, $p = 0.509$,

Allelic group: χ^2 =2.88, df = 3, p = 0.411; ERA group: χ^2 =5.07, df = 3, p = 0.167). There were no other genotype biases on the scrapie-affected farms (Risk group: χ^2 =6.22, df = 4, p = 0.183; ERA group χ^2 =1.51, df = 3, p = 0.679).

Figure 4.6 The proportions of sheep in each Allelic group found dead of unknown causes on scrapie-affected farms (sc) and scrapie-unaffected farms (c). 0 – represents the proportion of the flock which has not died of unknown causes; 1 – the proportion of the flock which has died of unknown causes.



4.4 Discussion

Only female sheep were included in this analysis of the fates data, as there was only limited information on male animals, which are more likely to be culled than female sheep, in line with the current sheep husbandry practices. Most female lambs are

usually retained on the farm as replacement breeding stock, while most male lambs are sold on for slaughter, with very few being retained for breeding.

In the Blind Cull Study, despite the trends observed in table 4.3 which suggested that sheep susceptible to scrapie were more frequently culled, with all the farms pooled together, no associations between genotype and being culled were found. This was also the case, when focussing on a pool of the four farms with culling percentages greater than 10 percent. *PrP* genotype significantly affected the fit in two models assessing the association between genotype and being culled, but only in one were the odds of being culled different to one. This was on a single scrapie-affected farm where sheep resistant to scrapie were being preferentially selected for culling, which suggests some selection against resistant genotypes.

It was possible that the performance of sheep of susceptible genotypes would be lower on scrapie-affected farms due to subclinical scrapie, and that more of these sheep would be selected for culling, as a result of poorer performance. However, this was not the case. In all the analyses on the pooled farms, the scrapie status of the farm was not associated with the culling decisions made by farmers and there was no association with genotype; whereas on the single scrapie-affected farm, sheep encoding ARR/ARR were most likely to be culled. This suggests, apart from that one farm, that farmers do not detect differences in productivity and the performance of susceptible sheep is similar to their more resistant counterparts on the farm.

Generally, among female sheep, it is expected that the risk of being culled from the flock would increase with age, with older ewes more likely to be culled due to poor reproductive performance and ill-health; as well as for welfare reasons when they are not thriving at pasture. In the analysis of the combined dataset, no associations between YoB and being culled were found which suggests that age was not overtly affecting the performance of the sheep. However, when only the farms with a culling level above 10% were analysed, an association with age was present: that older animals were more likely to be selected for culling, and when the farms were considering individually, is was seen that on two farms, there was an increased number of younger sheep being culled. These effects were masked by the culling patterns on the other farms in the original analysis, and in the Survivorship study where each farm was analyzed separately, associations between age and survival were more apparent. The associations with YoB on Farms A35 and U29 suggest that there was a number of sheep sampled in the first visit, which were subsequently sold on, instead of being retained as breeding replacements.

Overall, these results suggest that it is unlikely that there had been past selection for susceptible genotypes, and supports the findings in Chapter 3 (that farmers cannot determine the susceptibility of their sheep based on performance) as all genotypes were equally likely to be selected for culling. A farmer's decision to cull their sheep is not related to their genotype, and although age does appear to have an association with being culled, this is not present on all farms.

For the Survivorship Study, sheep which had died from scrapie were excluded to investigate the effects of genotype on survival in the absence of clinical disease. The overall trends observed in table 4.6 (that sheep susceptible to scrapie are more likely to be lost from the farm) were confirmed in eight of the farms. On these farms, the risk of being lost from the flock increases with increasing susceptibility to scrapie, on both scrapie-affected and scrapie-free farms. This is an expected finding, as the farmer knew the genotypes of the sheep, and the farmer could select for resistant sheep in accordance with the then recommended guidelines (Dawson *et al.*, 1998). A possible reflection of a change in practice based on these guidelines is seen on Farm P27. Prior to knowledge of the genotypes, resistant sheep were more likely to have been culled on this farm (table 4.4), but once the genotypes were known, there was selection for resistant genotypes (Appendix 2). Otherwise, inspection of the hazards calculated for each farm does not suggest that there is any stronger selection against susceptible sheep on either scrapie-affected or scrapie-unaffected farms.

In this study, there was also an affect of age (YoB) on survival on seven of the farms, with older animals being more likely to be lost from the farms: a typical pattern, with increased risk of culling or death as age increases. Again, the risk of culling is increased with age through reduced performance, poor reproduction or ill-health. The large drops seen in figure 4.3a may reflect yearly management culling. On Farm U29, however, this was not the case (as seen in the Blind Cull Study) with a large number of younger ewes being at higher risk of being culled (figure 4.3b; also see Appendix 2). This suggests that a large number of lambs were sampled, which were subsequently sold on for culling and is a feature particular to this farm.

Studies have shown that on scrapie-affected farms the survival of sheep highly susceptible to scrapie is lower than that of more resistant sheep, regardless of an animal's disease status (Chase-Topping *et al.*, 2005). This is due to the reduced lifespan of susceptible sheep on these farms, and the subsequent reduction in the number of offspring produced. This study has also shown that survival of sheep susceptible to scrapie is reduced, on both scrapie-free and scrapie-affected farms, but in a commercial flock, this is more likely to be due to farmers selecting against susceptible sheep once the genotypes are known.

The Found Dead Study compared the proportions of sheep which had died on the farm from both known and unknown causes. Similar to findings by Baylis *et al.* (2000) and McLean *et al.* (1999), a higher proportion of deaths from unknown causes (compared to those of known causes) occurred on scrapie-affected farms. This higher proportion of deaths appears unlikely to be entirely due to subclinical scrapie as this proportion included both resistant and susceptible genotypes (with a higher proportion of these sheep found dead of unknown causes encoding the VRQ allele) although the presence of atypical scrapie cannot be ruled out in resistant sheep. This does suggest there might be some subclinical disease present, with sheep not developing the typical clinical signs before dying. This is similar to results of a study in the Shetland Islands in which a high proportion of sheep found dead of unknown causes were found to have scrapie at post-mortem testing (Clark *et al.*, 1994; Humphry *et al.*, 2004),.

This chapter has shown that, even in the absence of clinical disease, there may be some natural selection against susceptible genotypes on scrapie-affected farms. However, there is no evidence to suggest that any particular genotypes were being preferentially selected by the farmer for culling prior to the advent of *PrP* genotyping. This suggests that historically, farmers have not been selecting for susceptible genotypes, and thus not contributing to the continued presence of scrapie-susceptible animals in the UK national flock and subsequent persistence of scrapie.

5 Longitudinal Lamb Study

5.1 Introduction

This longitudinal study was designed to follow the progress of a cohort of lambs over a course of a year, and investigate any potential relationships between *PrP* genotype and the lambs' weights and growth rates. The study followed their progress from birth to maturity on a selected farm, and involved close association with that farm.

For this study, information on both the individual lamb weights and the management systems of the flock could be obtained for inclusion in the analysis. Accurate paternities were available for the lambs, as well as information on their immediate environment, so any differences in lamb weights which may have been due to the differences in performance between their sires, or between the environments where they were raised, could be accounted for during the data analysis.

Unlike the studies performed in the other chapters, there is more detailed information on each sheep than just the productivity measures. Apart from the weights collected over the course of a year, there was also Signet data (which includes muscle and fat depths, as well as Estimated Breeding Values – EBVs – see Chapter 6) available for these lambs, as well as for other sheep present in the flock. This chapter considers both the lambs' weights under study and their Signet data; as well as the Signet data for all sheep present on the farm.

5.2 Materials and Methods

5.2.1 Recruitment

For this study, several criteria were used for farm selection. The farm had to be scrapie-free, so that subclinical disease would not confound any potential genotype effects on production; the farm had to have a large number of lambs available for study, and most importantly, the farmer had to be willing to commit a certain amount of time to the study and provide assistance where necessary, with appropriate handling facilities for weighing and blood sampling the lambs. It was also important that the farmer had not previously undertaken genotyping to avoid potential bias towards resistant genotypes on the farm.

There were a number of farms present in the IAH farm database (see Chapter 4) which had not been involved in the UK wide farm study (the IAH field-based scrapie study, described in Chapter 2). Using this database, a mailout was sent out to all farms known to be scrapie-free, and from the responses, a farm was chosen which fitted the above criteria.

The farm selected was a hill farm in Cumbria which specialised in the breeding and genetic improvement of Swaledale sheep; and sold their crossbred animals for the meat industry. The genetic improvement was mainly focussed on improving the breed overall, in terms of mothering ability, lamb numbers and survival, and overall hardiness, but was also beginning to consider *PrP* genotype, as the farm had joined the National Scrapie Plan's Ram Genotyping Scheme (DEFRA, 2005b) in January 2003, with around 400 sheep having been genotyped between January 2003 and

January 2004. Around 135 of the ewes and all the rams which would be producing the lambs for the study had been genotyped prior to the beginning of the study. This contradicted the selection criteria, but was only discovered after an agreement was made to work with this farm, and after the work had begun on this farm.

The Swaledale part of the flock consists of about 300 ewes which are extensively managed and reared on two areas of the hill farm: Green Farm and Rakehead. These ewes are mated yearly to a selection of rams, depending on the age of the ewe. Younger ewes (up to about four or five years of age) are mated to homebred Swaledale rams to produce breeding replacements (females and some males), and lambs for slaughter (males). Older ewes are mated to Border Leicester and Texel rams, to produce lambs for slaughter. This study followed the progress of the 2004 cohort of purebred Swaledale lambs over the course of a year.

5.2.2 Lamb weighing

Lambs were weighed four times: at birth, at around eight weeks of age, at around seven months of age (designated scan weight) and at around 11 months of age (designated mature weight). A digital spring balance was used measure the lambs' birth weights. The balance was suspended with a bucket attached and once the balance was zeroed, the lamb was placed in the bucket and the weight recorded. The lamb's ear tag number and sex were also noted, as well as whether it was a single, twin or triplet. Figures 5.1a and b are photographs of newborn lambs involved in the study.

Figure 5.1 a) Newborn Swaledale ewe lamb; b) Swaledale ewe tending her newborn twin lambs

(a)

(b)

A digital weigh crate was used to weigh the lambs at the other time points: eight weeks of age, at seven months and at 11 months of age. The lambs were gathered in a pen adjacent to the weighing crate, and then run through the crate individually,

recording both the weights and the ear tag numbers. Figures 5.2a and b show lambs at around eight weeks of age.

Figures 5.2a and b Lambs at approximately 8 weeks of age, and their dams

(a)

(b)

5.2.3 PrP *genotyping*

The method used to genotype the lambs is described in Chapter 2. Only seven genotypes were present, so different genotype groupings were used (table 5.1 cf. table 2.1). These groupings are similar to those already described: the main differences are that there are no sheep of Risk group 5 or ERA group d present; and that the Allelic grouping focuses on the presence or absence of the ARR allele, as there was a low number of sheep encoding the VRQ allele present.

Table 5.1 Genotypes of sheep present in the study flock and their mapping to susceptibility groups

ARR/ARR	1	ARR present	a
ARR/AHQ	2	ARR present	b
AHQ/AHQ	2	ARR absent	c
ARR/ARQ	3	ARR present	b
ARQ/AHQ	3	ARR absent	c
ARR/VRQ	4	ARR present	c
AHQ/VRQ	4	ARR absent	b

5.2.4 Analytical methods

The data were analysed using ANOVA and mixed modelling, and a 5% significance level was used. The response variables were lamb weights recorded for the study, and the Signet data for all recorded sheep (table 5.2).

Table 5.2 The response variables and the sources of the data for the analyses in this chapter. The Estimated Breeding Values include *8-week EBV*, *Scan weight EBV*, *Muscle depth EBV*, *Fat depth EBV*, *Maternal ability EBV*, *Mature size EBV*, *Litter size EBV* and *Index*.

	Number of animals available (number with genotype information)	Response variable	Data source
2004 lamb cohort	51 (16)	Birth weight	Field study
	267 (110)	8-week weight	Field study
	147 (136)	Scan weight	Field study
	130 (118)	Mature weight	Field study
	147 (136)	Muscle depth	Signet
	147 (136)	Fat depth	Signet
	288 (137)	Estimated Breeding Values	Signet
All other sheep	387 (233)	8-week weight	Signet
	326 (293)	Scan weight	Signet
	326 (293)	Muscle depth	Signet
	326 (293)	Fat depth	Signet
	842 (504)	Estimated Breeding Values	Signet

Table 5.3 summarises the explanatory variables included in the study. These variables include year of birth (YoB, which was not included in the lamb analysis as this only focussed on lambs born in 2004); sex, grazing area (the area of hill the sheep was raised on and grazes, either Green Farm or Rakehead); number of lambs born in the litter (LSB, singles vs. non-singles); sire (Sire ID), sire genotype and lamb genotype (Risk group, Allelic group and ERA group). Sire genotype and lamb genotype were evaluated in separate models.

Table 5.3 Summary of explanatory variable used in the analyses in the study

	Included in the analysis of:	
Explanatory variable	2004 lamb cohort	All other sheep
YoB	N	Y
Sex	Y	Y
Grazing area	Y	N
LSB	Y	Y
Sire ID	Y	Y
Sire genotype	Y	Y
Lamb genotype	Y	Y

In all analyses on the 2004 cohort of lambs, Sire ID was designated a random effect to partially account for the family structure. As detailed paternity was known for these lambs, and only a limited number of rams was used to sire the lambs, Sire ID was included in the analysis of all the lamb data, both of phenotypical and EBV data. The analysis of phenotypic measurements from the 2004 lamb data also accounted another family effect: multiple births among the ewes. As only a limited number of ewes had multiple births, dam could not be included as a random effect. Instead one lamb from each ewe with multiple lambs was selected at random for inclusion in the analysis. Multiple births were not accounted for in the analysis of the EBVs from the 2004 lamb cohort as EBVs are already calculated to take into account multiple births; nor were they accounted for in the analysis of the whole flock's Signet data.

In the analysis of the 2004 cohort of lambs, the term grazing area was compared individually to the response variables using mixed modelling with Sire ID as a random effect. If this term was found to be significant (at the 5% level), the model was refitted with this term as a random effect, and grazing area being nested within Sire ID, as the rams were used to cover sheep on both grazing areas.

In the analysis of the whole flock's Signet data, Sire ID was also included as a random effect as in earlier analyses in this chapter, but grazing area was not included in this part of the study as accurate information on this possible explanatory factor was not available for all the sheep.

5.2.5 Analysis 1 – lambs' weights and Signet data

Linear mixed models were used to examine the effect of each explanatory variable (table 5.3) on each of the response variables (weights and Signet data; table 5.2). In the analysis of 8-week weight, scan weight, mature weight and the Signet phenotypic measurements the age of the lamb was included as a covariate in the model. This was to allow for variations in age between the lambs. Where a significant effect of genotype groups was identified, Fisher's least significant differences (LSD) test was used to identify differences amongst susceptibility levels. This test is a pair-wise comparison technique and reduces Type I error.

5.2.6 Analysis 2 – lambs' overall growth patterns

Linear mixed models were used to analyse the overall pattern of growth of each lamb. The response variable is a sequence of measurements of weight (the overall growth pattern for each lamb) and the explanatory variables were those listed above in table 5.3 as well as the age of the lamb (in days) at each observation (birth, eight weeks, scan weight and 11 months of age). Age was retained in all the models, as well as retained any explanatory variable found to be significant at the 5% level.

Repeated measurements on each lamb over a period of time were analysed by including age grouped by lamb as a random effect, a method described by Crawley (2004), pages 685 - 689. This was necessary, as the repeated measurements on each lamb would result in temporal pseudoreplication and autocorrelation. This method of analysis accounts for differences in growth rates between successive time points, and for the non-independence of each of the measurements (Crawley, 2004).

5.2.7 Analysis 3 – all sheep Signet data

This analysis was similar to that described in section 5.2.5, except the term grazing area was not included because this information was not available for all the sheep. Sire ID was included as a random effect. In the analysis of the weights, muscle and fat depths, the parameters were divided by the age in days, rather than age fitted as a covariate, as described in the analyses in Chapter 6. This method was adopted for this analysis as accurate ages or dates of birth were not available for all the sheep represented by the Signet data. Each of the Signet parameters was compared to the explanatory variables in table 5.3 using mixed modelling. Table 5.4 provides a summary of each analysis; the data involved and variables included as random effects in the mixed modelling.

Table 5.4 Statistical techniques used in each of the analyses performed in this study, and the random effects included in the mixed models, where applicable.

	Response variable(s)	Random effects
Analysis 1	Lamb birth weight	Sire ID
	Lamb 8-week weight	Sire ID
	Lamb scan weight	Sire ID
	Lamb mature weight	Sire ID
	Lamb Signet data	Sire ID, grazing area
Analysis 2	Sequential lamb weight measurements	Age, sire ID, grazing area, lamb ID
Analysis 3	Flock Signet data	Sire ID

5.3 Results

5.3.1 Flock demography and genotype information

Most of the sheep (69.5%) represented by the Signet data were born in 2003 and 2004 (figure 5.3), including the information from the lambs monitored in the study. Most of the sheep were female, with only about 22.2% of the recorded flock representing males. The genotype distribution of these sheep by each of the genotype groups are given in figures 5.4a – c. The genotypes present show a predominance of more resistant *PrP* genotypes.

Figure 5.3 The birth cohorts represented by the Signet data

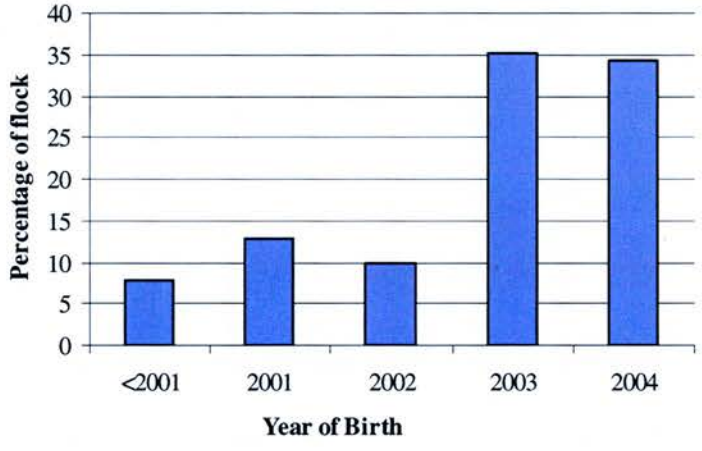


Figure 5.4 The genotypes of the sheep represented by the Signet data (a) Risk group; (b) Allelic group; (c) ERA group

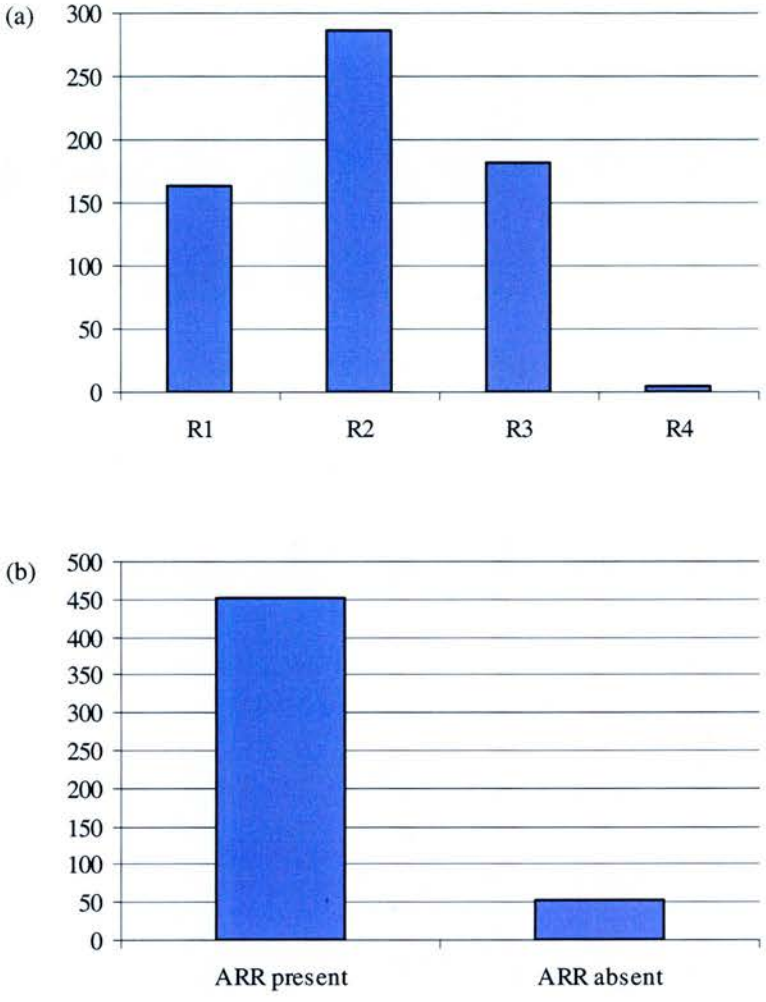
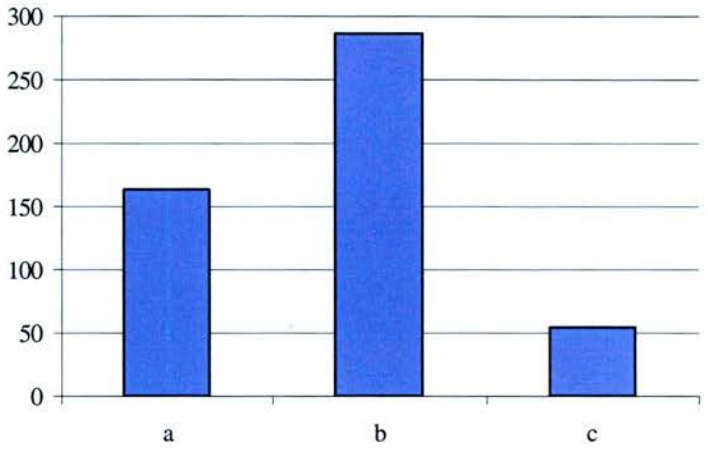


Figure 5.4 (cont)

(c)



Sire information is also available for this flock (figure 5.5). Most of the flock (87.4%) of the recorded flock were sired by three rams: B:28398 (42.9%, genotype ARR/AHQ), B:29398 (22.2%, ARR/ARR) and B:30160 (22.3%, ARR/ARR). The rest of the flock were sired by 14 other rams. Other possible evidence for breeding for scrapie resistance is that the majority of the sheep were sired by rams highly resistant to scrapie (figure 5.6).

Figure 5.5 The percentages of the sheep sired by each ram. The group labelled "other" represents 14 rams each of which sired ≤ 6 sheep (23 sheep in total).

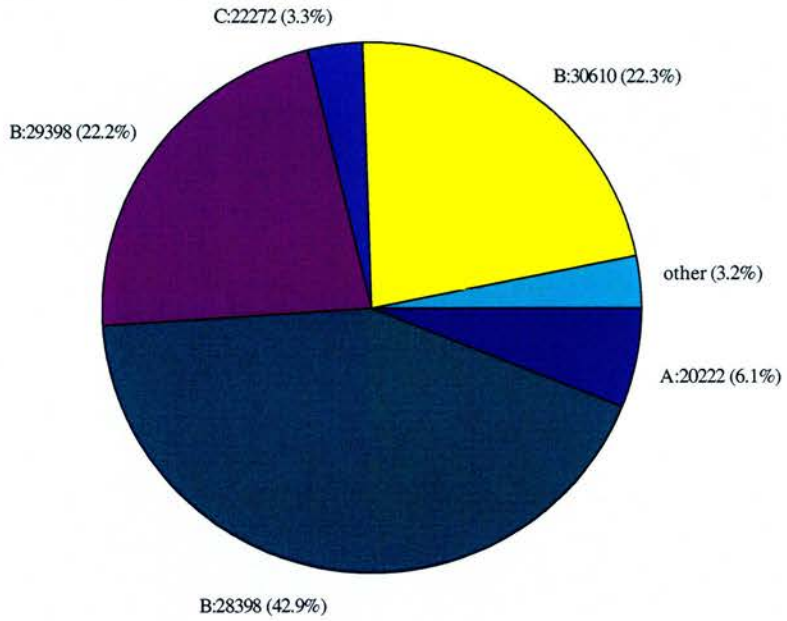
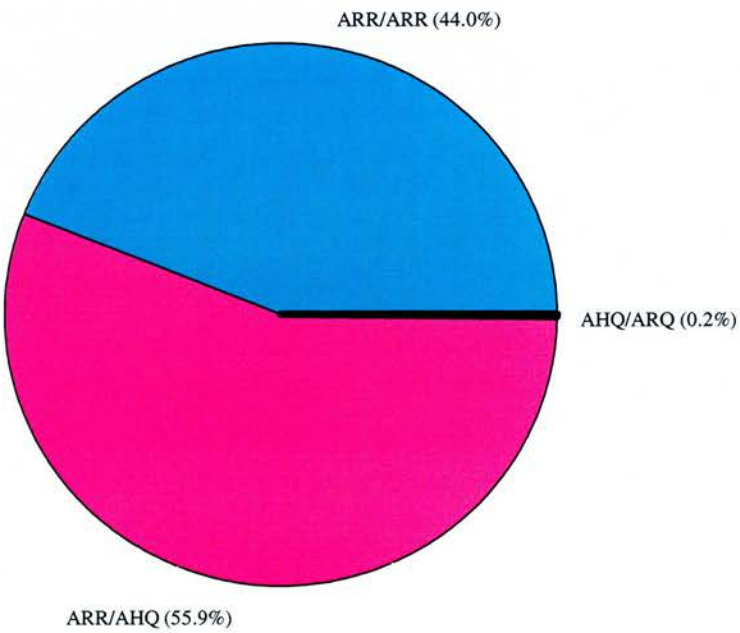


Figure 5.6 The genotype distribution of the rams used as sires.



The lambs were all born in March or April 2004. Most of the lamb data was for female sheep, with only about 30.2% male lambs recorded, as there had already been some pre-selection against the male lambs. Most males lambs were destined for meat and only males desired as tup replacements were accurately tagged and identified; and therefore useable in this study. The genotype distribution of the lambs is given in figures 5.7a – c, which show that the genotype distribution is skewed towards resistant genotypes, and no highly susceptible sheep are present.

Figure 5.7 The genotypes of the 2004 cohort of lambs (a) Risk group; (b) Allelic group; (c) ERA group

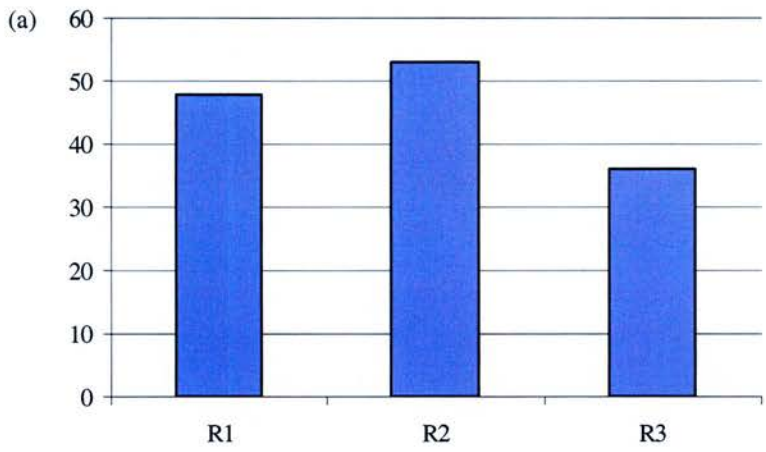
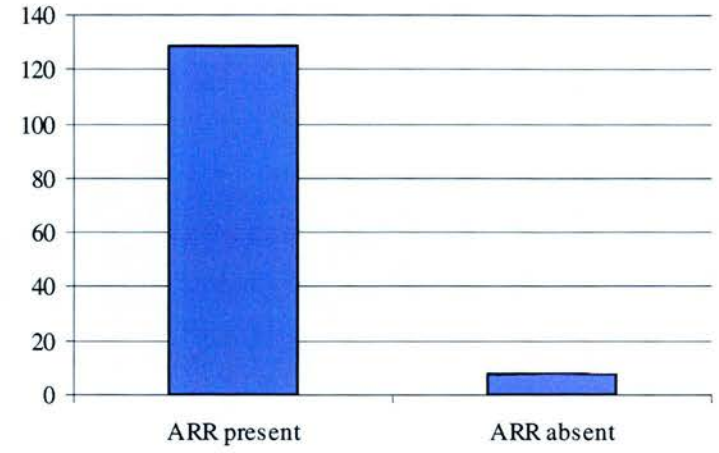
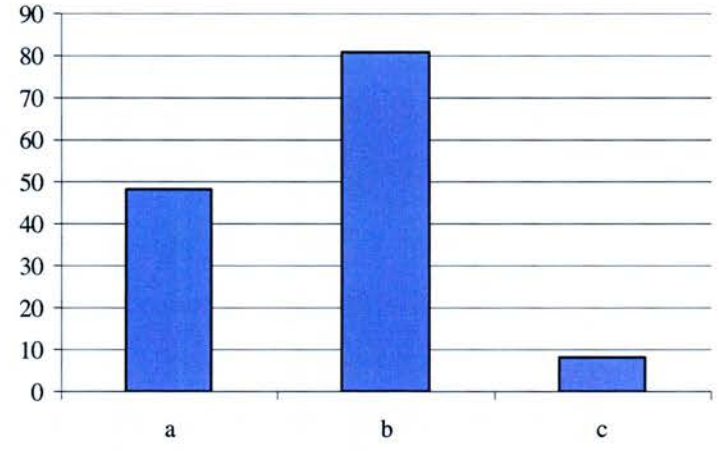


Figure 5.7 (cont.)

(b)



(c)



Four sires were used, one of which was not used to sire any lambs for breeding replacements (figure 5.8). Again, there is evidence of breeding for resistant lambs, as seen in the genotype distribution of the sires used, with 58.3% of the lambs sired by rams of the genotype ARR/ARR (figure 5.9).

Figure 5.8 The percentages of the lambs sired by each ram. The ram labelled White was only used to produce lambs for slaughter.

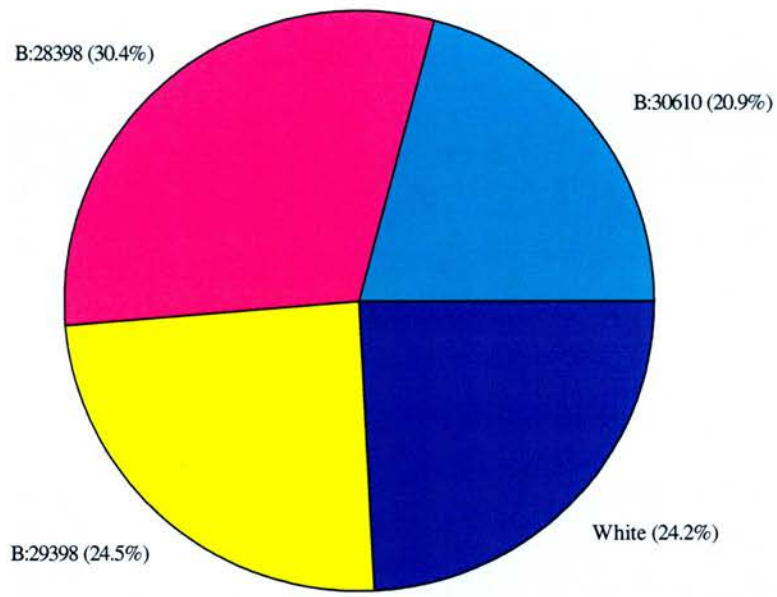
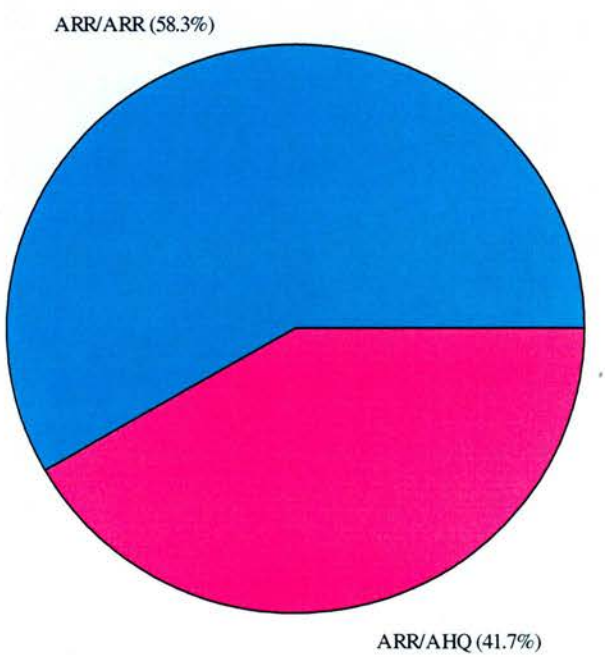


Figure 5.9 The proportions of the 2004 cohort of lambs sired by rams of each genotype



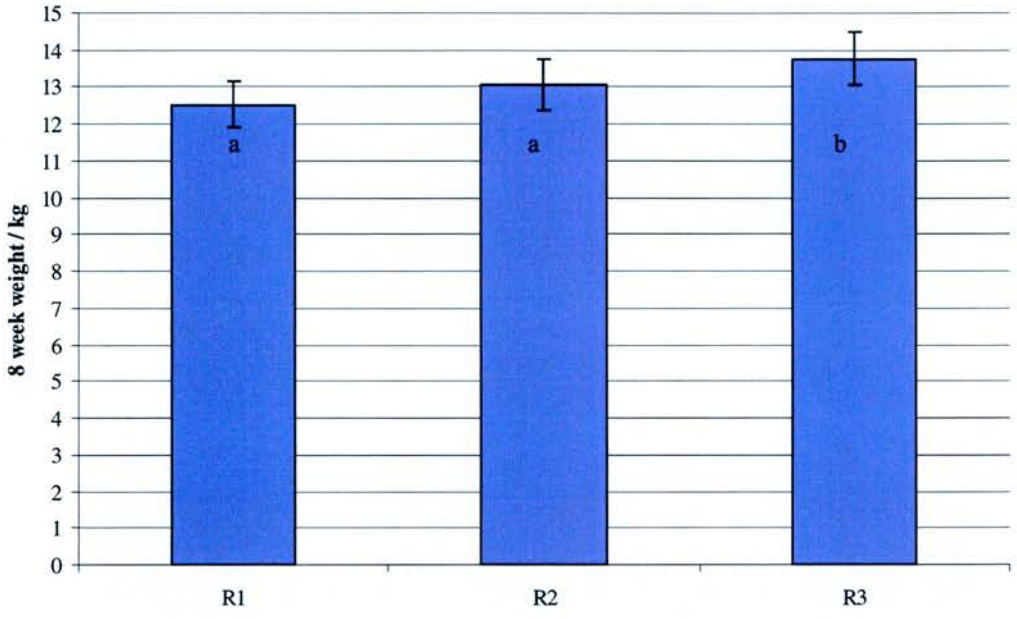
5.3.2 Analysis 1 – lambs' weights and lamb Signet data

Birthweight: In the fitted mixed model, there were no effects of sex, grazing area, lamb genotype or sire genotype on the birthweight of the lamb: only LSB significantly affected birthweight, with single lambs being about 0.8kg heavier than twins or triplets ($F_{1, 30} = 7.15$, $p = 0.012$).

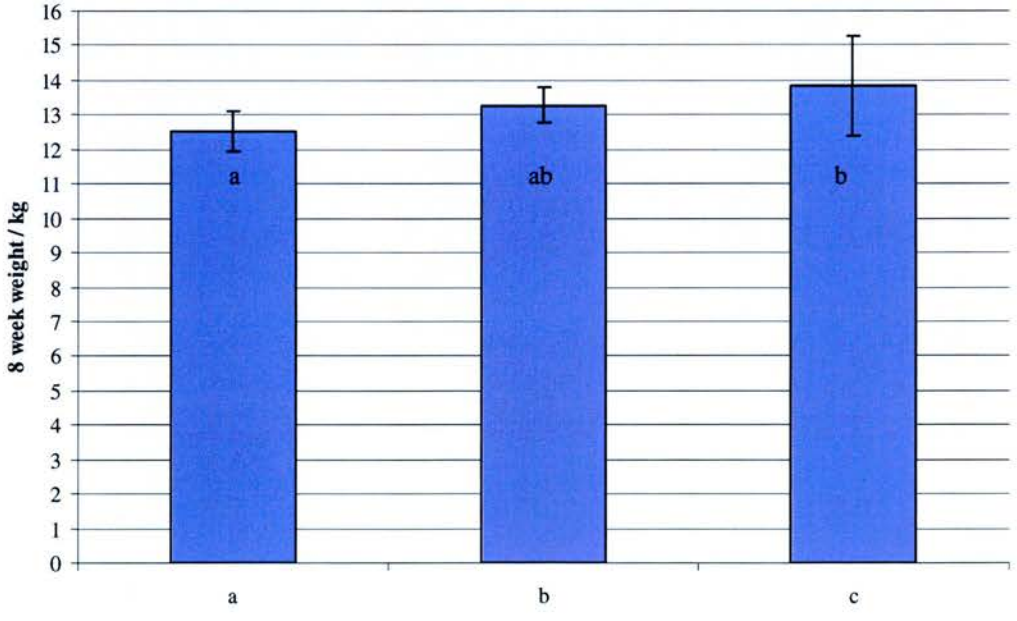
Eight week weight: With age fitted as a covariate, sex, LSB and lamb genotype were found to have a significant effect on 8-week weight. Males were found to be about 2.3kg heavier at eight weeks of age than females ($F_{1, 80} = 7.69$, $p = 0.007$); and single lambs were about 3kg heavier at eight weeks of age than non-singles ($F_{1, 80} = 58.83$, $p < 0.001$). Lamb genotype did have a significant association with growth rate at 8 weeks. Lambs in Risk group 3 had a higher eight week weight than those in risk groups 1 and 2, ($F_{2,80} = 4.88$, $p = 0.010$, also by Fishers LSD test) (figure 5.10). There was also marginal significance for ERA group ($F_{2,80} = 2.61$, $p = 0.079$), with lambs in ERA group c being about 1.3kg heavier than those in group a ($p = 0.049$, also by Fishers LSD test, figure 5.10b). Fishers LSD test is a pair-wise comparison technique used to determine which susceptibility levels were significantly different from each other, and reduces Type I error.

Figure 5.10 The mean 8-week weight (kg) by a) Risk group and b) ERA group. Results labelled with different letters are significantly different (Fishers LSD).

(a)



(b)



Scan weight (around seven months of age): With age fitted as a covariate, there were no effects of grazing area, sire genotype or lamb genotype on growth rate at this

age: only sex and LSB affected growth. Similarly to previous results, males were heavier at than females, this time by about 11.7kg ($F_{1, 113} = 130.33$, $p < 0.001$); and singles about 3.4kg heavier than non-singles ($F_{1, 113} = 12.84$, $p < 0.001$) at scanning age.

Mature weight (around 11 months of age): With age fitted as a covariate, only LSB affected weight. Similarly to previous results, singles were heavier than non-singles, this time by about 1.5kg ($F_{1, 100} = 29.79$, $p < 0.001$). There were no males with a mature weight measurement.

As expected, there was a significant positive association with age in all the analyses where this was included as a covariate. Table 5.5 provides a summary of the significant associations found between weight and the explanatory variables.

Table 5.5 Summary of associations between lamb weights and explanatory variables found in analysis 1.

Response variable	Explanatory variable	p-value	Summary of effect
Birth weight	LSB	0.012	Singles > non-singles
8-week weight	Sex	0.007	M > F
	LSB	<0.001	Singles > non-singles
	Risk group	0.010	R3 > R1, R2
	ERA group	0.079	c > a
Scan weight	Sex	<0.001	M > F
	LSB	<0.001	Singles > non-singles
Mature weight	LSB	<0.001	Singles > non-singles

The significant associations between Signet parameters and the explanatory variables are given in table 5.6. There was no effect of the *PrP* genotype of the ram on any of

the productivity traits. All the EBVs were strongly associated with Sire ID ($p<0.001$), which was fitted as a random effect. The effects of sire were variable and are summarised in table 5.7. Generally, sire B:29398 appears to associated with higher mean EBVs than other the other sires represented. Grazing area was also fitted as a random effect where appropriate, and lambs reared on 'Green Farm' appeared to have higher EBVs which are associated with maternal ability than lambs reared on 'Rakehead'(mean *8-week EBV* : 0.06 cf. -0.04; *Maternal ability*: 0.02 cf. -0.05; and *Litter size EBV*: 0.002 cf. -0.009).

Table 5.6 Summary of the associations between the lambs' Signet response variables. Table 5.7 indicates the different effects of Sire on the various EBVs.

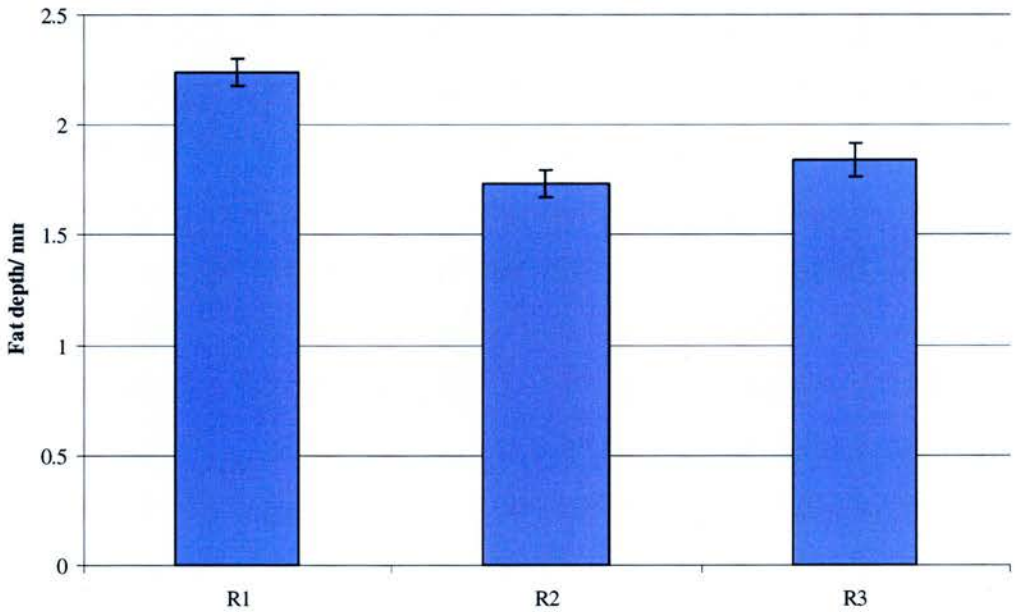
Response variable	Sire	Grazing area	Sex	LSB	Genotype group
Muscle depth	ns	ns	<0.001	ns	ns
Fat depth	ns	ns	ns	ns	Risk group 0.087 ERA group 0.089
8-week EBV	<0.001	0.042	ns	0.003	ns
Scan weight EBV	<0.001	ns	ns	-	ns
Muscle depth EBV	<0.001	ns	ns	0.005	ns
Fat depth EBV	<0.001	ns	ns	0.006	ns
Mature size EBV	<0.001	ns	ns	ns	ns
Maternal ability EBV	<0.001	0.047	0.002	ns	Allelic group 0.035 ERA group 0.062
Litter size EBV	<0.001	0.005	ns	0.012	ns
Index	<0.001	ns	ns	ns	ns

Table 5.7 The effects of Sire on the Signet EBVs.

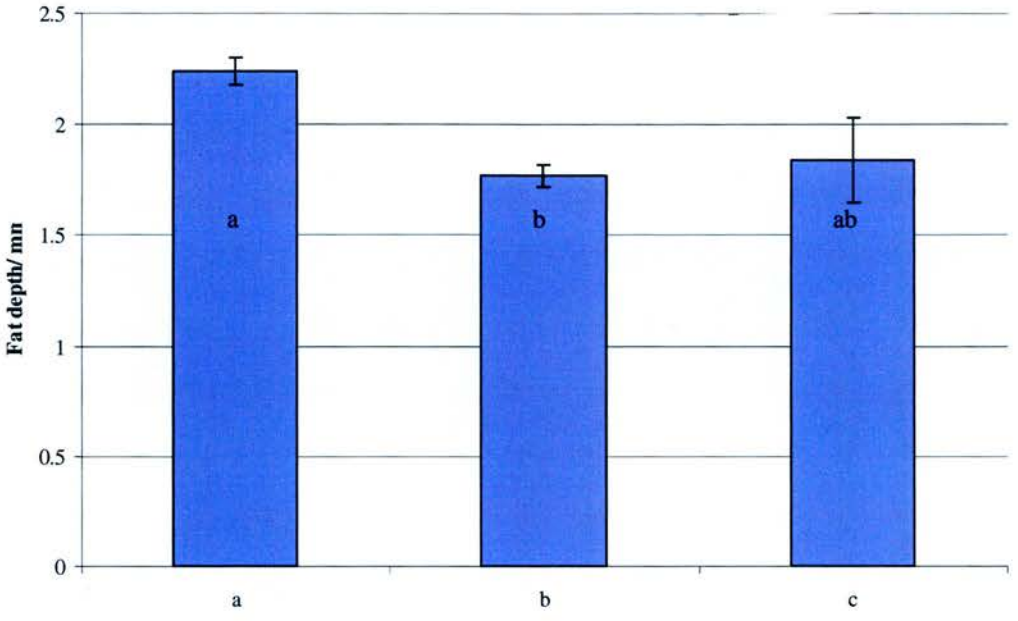
Signet value	Sire
8-week EBV	B:29398 > B:30160 > B:28398
Scan weight EBV	B:29398 > B:28398, B:30160
Muscle depth EBV	B:28398 > B:30160, B:29398
Fat depth EBV	B:29398, B:28398 >B:30160
Mature size EBV	B:29398 > B:28398 > B:30160
Maternal ability EBV	B:29398 > B:28398, B:30160
Litter size EBV	B:29398 > B:30160 > B:28398
Index	B:29398 > B:28398, B:30160

Muscle depth was only significantly associated with sex: males had about 3.3mm greater muscle depth than females ($F_{1, 114} = 32.70$, $p < 0.001$). There was a marginal association between genotype and fat depth. Under the risk grouping, R1 sheep had greater fat depths than R2 sheep ($F_{2, 106} = 2.50$, $p = 0.087$, figure 5.11a); and ERA group a sheep have greater fat depths than group b sheep ($F_{2, 106} = 2.47$, $p = 0.089$, figure 5.11b). In both cases, the different in fat depth is about 0.5mm. Again, as expected, muscle and fat depths were positively associated with increasing age.

Figure 5.11 The mean fat depth (mm) by a) Risk group and b) ERA group. Columns labelled with different letters are significantly different (Fishers LSD).



(b)

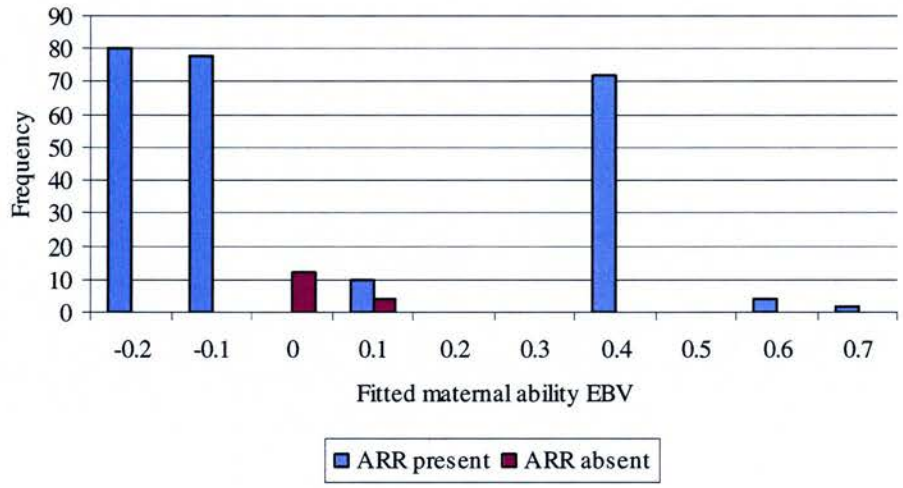


Males were found to have a higher mean *Maternal ability EBV* than female sheep (0.25 cf. -0.03). This result may be an artefact of calculation as this EBV is the maternal component of the *8-week* EBV, and males generally have higher eight-week weights than females. Single lambs were found to have a higher mean *8-week EBV* (0.13), but lower mean *Muscle depth* (-0.18), *Fat depth* (-0.05) and *Litter EBV* (-0.007), than lambs born as twins or triplets (-0.04, 0.03, 0.00 and -0.003 respectively). The last result (for *Litter EBV*) is expected as this EBV is based on the number of lambs born in a litter.

There appears to be an association between *Maternal Ability EBV* and lamb genotype. Lambs not encoding the ARR allele have a higher median *Maternal Ability EBV* than those which encode at least one ARR allele (-0.02 cf. -0.19, $F_{1, 123} = 4.52$, $p = 0.035$). These results suggest that when these lambs reproduce, those females not encoding ARR are more likely to have better mothering ability than those encoding

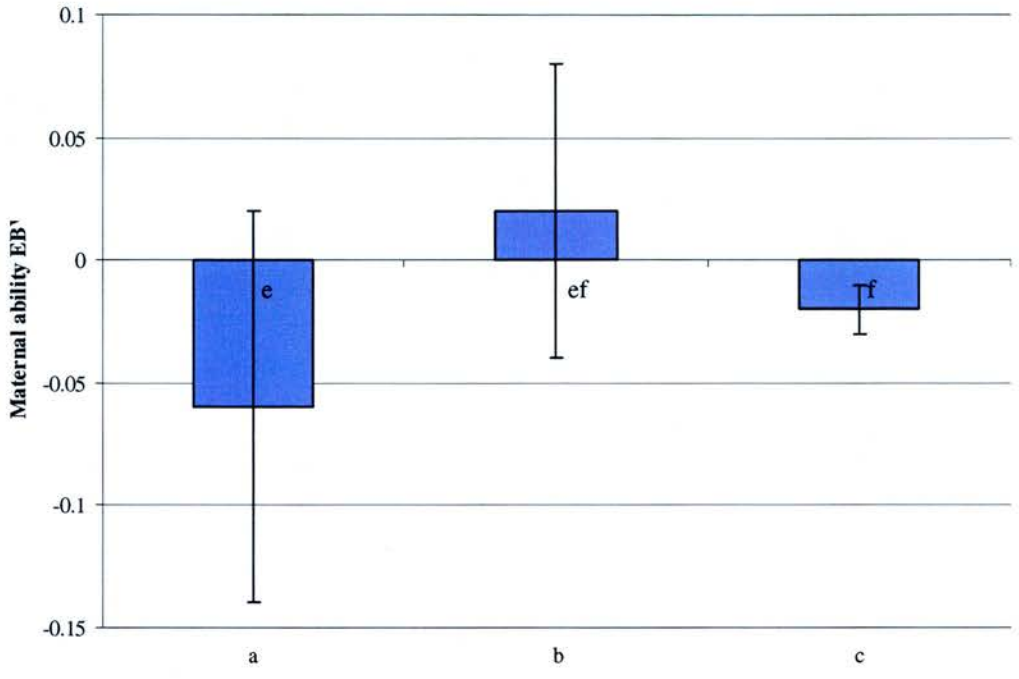
at least one ARR allele. This result appears to be reversed when considering the mean EBV. The mean *Maternal ability EBV* for lambs encoding the ARR allele is -0.01 (± 0.05), but is less for lambs not encoding the ARR allele (-0.02 ± 0.01). This is explained by considering the distribution of the fitted values. The fitted values for *Maternal ability EBV* for lambs encoding ARR are tending to be at the extremes of the scale, with either very high or low values, whereas those for lambs not encoding the ARR allele are more centrally distributed (figure 5.12).

Figure 5.12 The distribution of the fitted *Maternal ability EBVs* by the Allelic group of the lambs.



Under the ERA grouping, lambs in ERA group c had a greater *Maternal Ability EBV* than those in group a. This was a marginal association ($F_{2, 122} = 2.84$, $p = 0.062$), which also suggests that the ARR allele in these lambs is associated with a lower *Maternal ability EBV* (figure 5.13).

Figure 5.13 The mean *Maternal ability EBV* by ERA group. Columns labelled with different letters are significantly different (Fishers LSD)



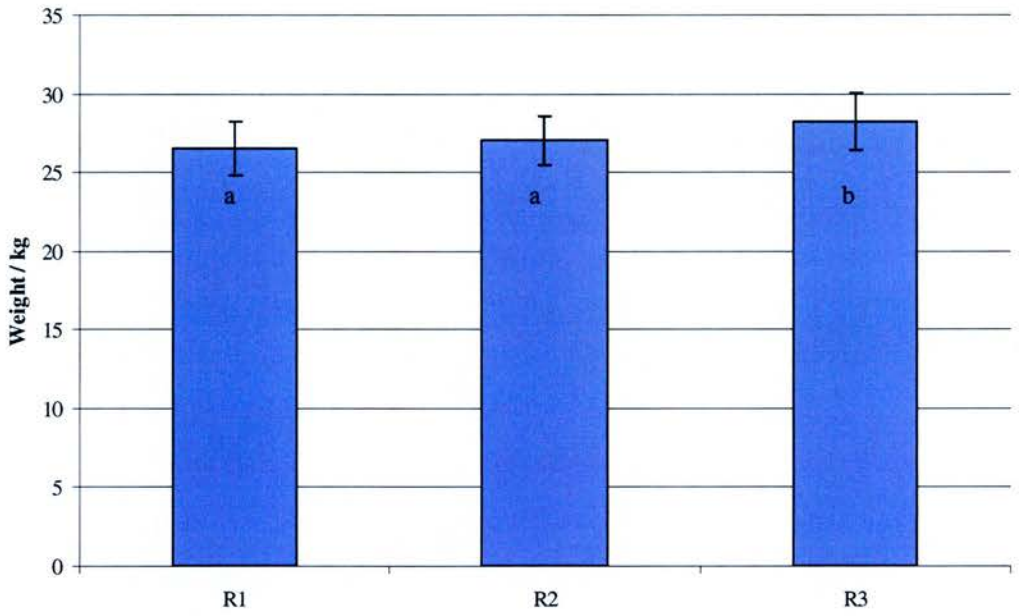
5.3.3 Analysis 2 – lambs' longitudinal growth patterns

Grazing area significantly affected the overall growth ($F_{1, 156} = 5.46$, $p = 0.021$), with lambs growing faster on Green Farm (mean: 27.16kg) than Rakehead (mean: 23.92kg), so this term was included as a random effect. Only the terms sex, LSB and age had any significant associations with the overall growth pattern. Males achieved heavier weights than females ($F_{1, 152} = 12.83$, $p < 0.001$) and singles were heavier than non-singles (27.14kg cf. 24.31kg; $F_{1, 152} = 38.95$, $p < 0.001$). Again, as expected, weight increased with age. There was a slight association between the longitudinal growth pattern and Risk group ($F_{2,97} = 2.71$, $p = 0.072$). Lambs in risk group 3 were found to be heavier than those in risk groups 1 and 2 (Fishers LSD, figure 5.14). All results are summarised in table 5.8.

Table 5.8 Summary of associations between the longitudinal growth patterns of the lambs

Response variable	Explanatory variable	p-value
Longitudinal growth pattern	Grazing area	0.021
	Age	<0.001
	LSB	<0.001
	Sex	<0.001
	Risk group	0.072

Figure 5.14 Mean weights of the lambs divided by risk group. Results labelled with different letters are significantly different (Fishers LSD).



5.3.4 Analysis 3 – all sheep Signet data

Although sire was fitted as a random effect, not all the sires were included: the group labelled 'other' (figure 5.5) only represented 23 sheep, and was excluded from the analysis. There were no significant relationships between genotype and the Signet data in this analysis. Sex, YoB and LSB predominantly affected weights, muscle and fat depths; and Sire was most strongly associated with the EBVs (table 5.9).

Table 5.9 The p-values for the significant associations between Signet data and various explanatory variables.

	Sire	Sex	YoB	LSB	Genotype
8-week weight	ns	ns	<0.001	<0.001	ns
Scan weight	ns	<0.001	<0.001	<0.001	ns
Muscle depth	ns	<0.001	<0.001	0.002	ns
Fat depth	ns	ns	0.002	0.014	ns
8-week EBV	<0.001	ns	ns	0.002	ns
Scan weight EBV	<0.001	ns	0.048	ns	ns
Muscle depth EBV	<0.001	ns	ns	ns	ns
Fat depth EBV	<0.001	ns	ns	ns	ns
Mature size EBV	<0.001	ns	0.008	ns	ns
Maternal ability EBV	<0.001	ns	0.003	ns	ns
Litter size EBV	<0.001	ns	<0.001	<0.001	Risk group: 0.072
Index	<0.001	ns	<0.001	ns	ns

Sex had a significant relationship with *Muscle depth* and *Fat depth*. Male sheep had a slighter greater scan weight (per day of age) than female sheep (0.18 kg/day compared to 0.16kg/day), although muscle depth was slightly lower (males: 0.09 cf. females 0.10mm/day). Lambs born as singles also have greater *8-week weight* (0.31kg/day), *Scan weight* (0.18kg/day), *Muscle depth* (0.102mm/day) *Fat depth* (0.096kg/day) and *8-week EBV* (0.11) than those born as twins or triplets (0.26kg/day, 0.16kg/day, 0.098mm/day and 0.008mm/day, 0.035, respectively).

Values for *8-week weight, Scan weight, Muscle depth* and *Fat depth* were available only for the years 2003 and 2004, with all mean measurements being higher in 2003. Other variables with an association with YoB are *Scan weight EBV*, *Mature size EBV*, *Maternal ability EBV*, *Litter size EBV* and *Index.* The magnitude of these effects is given in table 5.10.

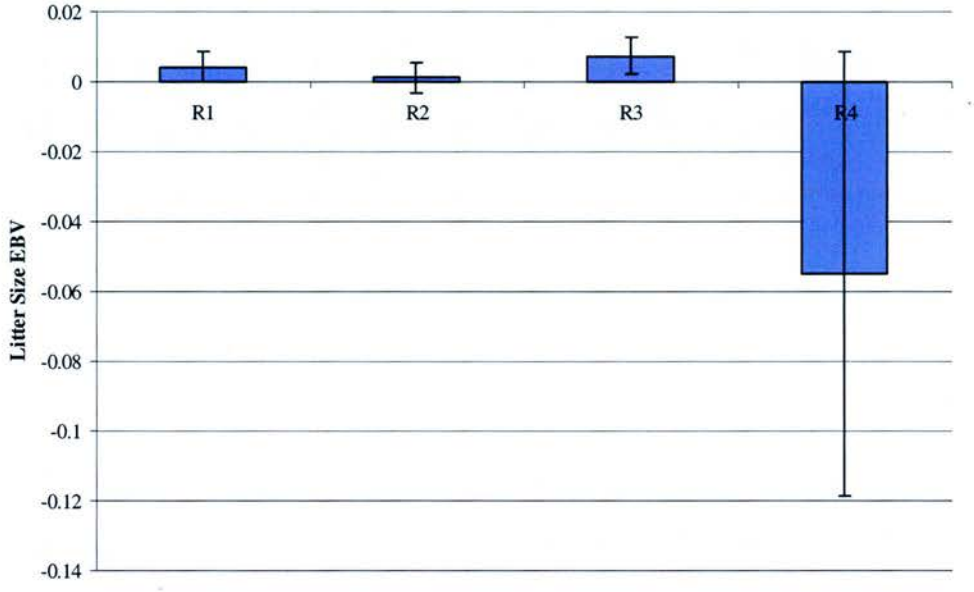
Table 5.10 A summary of the magnitude of the effects of YoB on each of the Signet parameters.

	8-week weight	Scan weight	Muscle depth	Fat depth	Scan weight EBV
<2001	-	-	-	-	-
2001	-	-	-	-	-
2003	0.28	0.19	0.12	0.010	0.28
2004	0.26	0.13	0.07	0.008	0.11

	Mature size EBV	Maternal ability EBV	Litter size EBV	Index
<2001	-	-0.03	-	-
2001	0.22	0.08	-	118.98
2003	0.49	0.04	0.01	130.78
2004	0.26	-0.02	0.00	119.90

Litter size EBV was also associated with the number of lambs born in the litter (an expected result, as *Litter size EBV* is a function of the number of lambs born in a litter), with non-singles having a higher mean EBV than single lambs (0.012 compared to 0.003). This variable was also marginally associated with the Risk group of the lamb. Sheep in risk groups 1, 2 and 3 all had higher *Litter size EBVs* than those in Risk group 4 ($F_{3,295} = 2.35$, $p = 0.072$) (figure 5.15).

Figure 5.15 The associations between Risk group and *Litter size EBV*. The values for R1, R2 and R3 sheep are not significantly different from each other, but are significantly greater than the value for R4 sheep (Fishers LSD).



The different associations between each Signet parameter and the explanatory variables sex, YoB, LSB and Sire are summarized in table 5.11. This table also summarised the effects of the sires. These effects are variable, although sire A:20222 appears to be associated with higher EBVs.

Table 5.11 Sex, YoB, LSB and Sire associations with each Signet trait.

Signet trait	Sex	YoB	LSB
8-week weight	-	2003 > 2004	Singles > non-singles
Scan weight	M > F	2003 > 2004	Singles > non-singles
Muscle depth	F > M	2003 > 2004	Singles > non-singles
Fat depth	-	2003 > 2004	Singles > non-singles
8-week EBV	-	-	Singles > non-singles
Scan weight EBV	-	2003 > 2004	-
Muscle depth EBV	-	-	-
Fat depth EBV	-	-	-
Mature size EBV	-	2003 > 2001, 2004	-
Maternal ability EBV	-	2003 > '<2001', 2001, 2004	-
Litter size EBV	-	2003 > 2004	Non-singles > singles
Index	-	2003 > 2001, 2004	-

Table 5.11 cont.

Signet trait	Sire
8-week weight	-
Scan weight	-
Muscle depth	-
Fat depth	-
8-week EBV	B:29398 > B:30610 > A:20222, C:22272 >B:28398
Scan weight EBV	A:20222, B:29398 >C:22272 > B:28398, B:30610
Muscle depth EBV	A:20222 > C:22272 >B:28398 > B:29398, B:30610
Fat depth EBV	A:20222, C:22272 > B:28398, B:29398 > B:30610
Mature size EBV	A:20222, B:29398 >C:22272 > B:28398, B:30610
Maternal ability EBV	B:29398 > A:20222 >B:28398, B:30610, C:22272
Litter size EBV	A:20222 > B:29398 > C:22272 > B:30610 >B:28398
Index	A:20222 > B:29398 > C:22272 > B:30610 >B:28398

5.4 Discussion

Although the farm selected did initially appear to meet all the criteria (scrapie-free, large numbers of lambs, good farmer assistance, good facilities, no bias towards resistant genotypes), it became apparent soon after the study began that there had been more genotyping work performed that originally indicated on the recruiting questionnaire and that there was going to be a bias towards resistant genotypes, and that there was only a limited number of lambs (~200) available to work with. Additionally, there were a reduced number of genotypes present, as this farm had been de-stocked as a result of the 2001 UK Foot-and-Mouth Disease epidemic; and the flock had been restocked by sheep of more resistant genotypes so no highly susceptible sheep were present. While the reduced number of lambs present would be expected to reduce the power of this study, the small range of genotypes present in part counteracts this, and each genotype was well represented within the flock. To compensate for the lack of sheep encoding the VRQ allele, the study was focussed on a comparison of the performance of ARR/ARR sheep with sheep encoding other alleles, such as AHQ and ARQ.

The study of a cohort of lambs over a year has revealed a very slight association between *PrP* genotype and lamb weight and overall growth patterns. At eight weeks of age, lambs in risk groups 1 and 2 (genotypes ARR/ARR, ARR/AHQ and AHQ/AHQ) were found to have significantly lower weights than those in risk group 3 (genotypes ARR/ARQ and ARQ/AHQ). The average difference in weights between these risk groups was between 0.5kg and 1kg (figure 5.10a). A similar trend is apparent with the ERA grouping, although this model was not significant at the 5% level. Sheep in ERA group c (genotypes AHQ/AHQ, ARQ/AHQ, and ARR/VRQ) were on average about 1.5kg heavier at eight weeks of age than those in ERA group a (ARR/ARR) (figure 5.10b). The model evaluating overall growth pattern indicated that lambs in risk group 3 would be between approximately 1kg and 1.5kg heavier than those in risk groups 1 and 2 on average (figure 5.14). These differences in weight are very slight between the genotypes and are unlikely to be easily detectable to the farmer, especially when compared to the mean weights (eight-week weight: around 4% to 8% greater; overall around 6% greater than the ARR/ARR genotype).

A similar pattern (indicating that the ARR allele was associated with poorer weights) was found in German Black-Headed Mutton sheep, although this result was dismissed by the authors as it was based on a study which compared 93 sheep encoding the ARR allele to six not encoding this allele (de Vries *et al.*, 2004b); and another study on Suffolk sheep also did not find any significant relationships between *PrP* genotype and the lean growth rate (Prokopová *et al.*, 2002).

One study has found an association between sex and birthweight, with males being heavier than females (Roden *et al.*, 2003), however in this study there was no effect of sex on birthweight: this was determined by the litter size. Single lambs have higher birthweights than lambs from multiple litters, presumably as singles have no competition for nutrients or space in the womb. The weights recorded at the other time points were mostly affected by sex and litter size, with males being heavier than females, and single lambs having higher weights than twins or triplets, and in the case of the overall growth pattern, there was a difference in the weights on the two grazing areas, which might reflect different qualities of pasture, a factor which would become more important to older lambs once they have been weaned.

The number of lambs present in a litter appeared to remain a limiting factor on the weight a lamb achieved up to the end of observation in this study. It was expected that as the lambs mature, the smaller lambs from multiple litters would 'catch up' with single lambs in weights and size, but this was not observed in the period of observation in this study (11 months), although the decrease in the differences between the weights of single and non-single lambs suggest that both groups of lambs would have the same average weight eventually.

No associations between muscle mass or depth and *PrP* genotype were found in East Friesian milk sheep and German Black-Headed Mutton sheep (de Vries *et al.*, 2004, 2005), and this was also the case in this study. Sex influenced muscle depth, with males, as expected, having a larger muscle depth than females. There was a marginal association between lamb genotype and fat depth, with lambs encoding ARR/ARR

(R1 and ERA group a) being associated with a higher fat depth. This is a very slight difference (about 0.5mm), which, similar to the findings on lamb weights, is unlikely to contribute to a farmer's perception of their sheep.

A lamb's EBV is very strongly associated with its sire. This is because very limited information is available directly from each lamb for calculating EBVs, so they are predicted from the performance of directly linked family members. There is only one significant relationship between lamb genotype and EBV, under the Allelic grouping, and one marginal association with the ERA grouping, which suggests that sheep more likely to be resistant to scrapie have lower *Maternal ability EBVs*. Otherwise, only sex, LSB and grazing area, had significant associations with EBV.

The EBVs for all the sheep are mainly influenced by sire which reflects the different breeding potentials among the different rams. These EBVs are also influenced by the YoB, with the year 2003, being associated with the higher mean EBV in many of the traits. The number of lambs born influenced *Litter size EBV*, with lambs born in multiple litter having higher EBVs than single lambs. *Litter size EBV* is a predictor of litter size, so this is to be expected. The number of lambs in a litter also influenced *8-week weight EBV*, with singles having greater mean EBVs on average. This might reflect that at this age, singles are heavier than non-singles. The association with sire also suggests that certain rams are more likely to sire larger litters. The other Signet measurements (*8-week weight, Scan weight, Muscle* and *Fat depth*) were influenced by sex, YoB and LSB. As previously observed, lambs born to multiple litters do not immediately appear to 'catch up' in size and weight with single lambs. *Litter size*

EBV was also marginally associated with Risk group, with the most susceptible group present having a lower mean EBV than other genotypes of sheep present: this result is also seen in the analysis of the Signet data of other flocks (Chapter 6, page 116), that is that susceptibility to scrapie is associated with poorer *Litter size EBVs*.

There appear to be two sets of conflicting results within the study. Firstly, in analysis one, single lambs have lower *Muscle depth* and *Fat depth EBVs* than lambs born into multiple litters, whereas in analysis three, single lambs have greater *Muscle* and *Fat depths* than those born in multiple litters. The results of analysis three are most likely due to the Sire influence on the EBVs, rather than reflecting the actual measurements of the lambs themselves (LSB was not found to have any significant association with *Muscle* and *Fat depth* measurements). Secondly, in analysis one, the analysis of the muscle depth*s* indicates that males had a greater muscle depth than females, whereas in analysis three, this finding is reversed. This latter result is like to be an artefact of the fitted model, as the fitted model muscle depths were very similar.

In summary, this longitudinal study on lamb growth has revealed that the weights are influenced by a number of external factors, the sex and number of lambs in a litter being the most important. However, there does seem to be some association with *PrP* genotype, and if this association had been sporadically observed on farms in the past, it may have led to the theory that scrapie-resistant animals do not perform as well as their susceptible counterparts, providing that the farmer was actually able to detect them: the results here suggest that without weighing devices, any differences present would not be overtly obvious.

6 Can we detect a relationship between *PrP* genotype and heritable traits of interest to sheep farmers?

6.1 Introduction

Sheep (*Ovis aries*) are thought to have been domesticated some 9000-11000 years ago in western Asia (Franklin, 1997), although their origins are unclear (Hiendleder *et al.*, 1998). Initially, domestic sheep were thought to mainly descend from mouflon sheep (*O. musimon*, *O. orientalis*) (Lush, 1945). Hiendleder *et al.* (1998) found that the mitochondrial DNA sequences of domestic and mouflon sheep are similar, which suggests a common, but unknown, ancestor or that mouflon were derived from early domestic breeds. Further work has confirmed that at least one line of domestic sheep was derived from mouflon, which was then introduced to Europe (Hiendleder *et al.*, 2002).

Domestication of sheep allowed for the survival of a number of variations (mutations) which would not have otherwise persisted under natural selection (Ryder, 1984). It also resulted in increased outbreeding as animals were introduced to new areas, and increased inbreeding as animals were confined to more restricted areas (Lush, 1945). Inbreeding could be deliberate or accidental. In the 18th century, inbreeding was found to be a way of generating new traits and was deliberately performed in some instances (Parry, 1983b). Accidental inbreeding occurred as the parentage of the animal was not usually known beyond a few generations, and so some sheep were mated to relatives (Lush, 1945). Some of the variation achieved by breeding and domestication produced traits which were desirable to man, such as the

different wool types, milk yield and growth rate/carcase composition. Selection for these traits has resulted in modern sheep breeds and their characteristics.

Today, most of these breeds and their characteristics are still subject to genetic improvement, although now the dangers of inbreeding are recognised, and generally avoided by farmers. Genetic improvements in sheep in the UK are mainly focussed on lamb and meat production, which involves the survival, growth rates and carcass characteristics of the lambs produced. All these traits have some measure of heritability, the proportion of phenotypic variation which is due to additive genetic variance (Cameron, 1997). Fogarty (1995) provides a review of all these heritabilities, calculated by different methods, for different breeds and includes the correlations between heritability and phenotype. Genotypic and phenotypic correlations between different characteristics, such as liveweight gain and wool production are also presented. The number of different methods used to calculate the heritability of each of these traits suggests the difficulty in producing a perfect way to estimate the heritable genetic merit of an animal. This is because most traits are influenced by a number of genes and the environment. There are a few traits known to be controlled by a single gene, such as the callipyge gene, which causes double muscling in sheep and the booroola gene, which results in increased litter size, but these are exceptional.

Currently, the most frequently used method to estimate the genetic merit of an animal for a particular trait is by using BLUP (Best Linear Unbiased Predictor, van Heelsum *et al.*, 2001). BLUP is an application of mixed modelling, a statistical technique which allows for errors and random effects associated with individual

observations, as well as accounting for correlations between observations which are introduced because of family structure within a flock (Henderson, 1950; Henderson *et al.*, 1959; Henderson, 1963; Henderson, 1974,1975; Schaeffer, 1991). Mixed models are fitted to the productivity data of a pedigree, assuming that genetic and environmental effects are additive (Henderson, 1949; van Heelsum *et al.*, 2001): this produces an EBV (Estimated Breeding Value) which is a numerical indication of the amount of heritable genetic merit. In the UK, sheep EBVs are calculated and provided by Signet Sheepbreeder®, a consultancy sub-division of the Meat and Livestock Commission, and are based on data collected from the farmer at key events throughout the year, such as tupping time and lambing season.

This chapter investigates any potential relationships between *PrP* genotype and certain phenotypic values (such as weights) and EBVs using ANOVA and pair-wise comparison techniques, while allowing for any potential confounding effects such as the time of the year when the sheep is born. This analysis involving EBVs allows for investigation as to whether certain genotypes of sheep are indeed more productive, when environmental factors and familial input are accounted for.

6.2 Methods

6.2.1 Data sources

The data used in this study was provided by Signet Sheepbreeder®, which has protocols for collecting the necessary information required to derive the EBVs. All farmers enrolled in the Signet scheme are required to submit the details of their flock: in the case of new clients, identities, sex and dates of birth of all their stock;

for established flocks, details of any replacement sheep brought into the flock. Two year-old ewes are weighed prior to tupping (generally at the beginning of November for a spring lambing season at the beginning of April), and these weights included in the Signet database. After the lambing season, farmers are required to submit details of which ewe was mated to which ram; and lambing details which include: litter size, date of birth and sex of the lambs. The lambs' individual weights are recorded at eight weeks and again at 20-21 weeks of age, and additionally, at 20-21 weeks of age, the muscle and back fat depths over the third lumbar vertebrae of each lamb are measured using ultrasound (Meat and Livestock Commission, 2005).

Signet uses this recorded information (weights, muscle and fat depth; date of birth, sex and family linkages to predict EBVs for individual traits using BLUP, three of which, Scan weight, Muscle Depth and Fat Depth EBVs, are used to produce an overall Selection Index score, to allow a breeder to select for several traits at once (Meat and Livestock Commission, 2005). In this study, the Selection Indices used are the Lean Index and a Scheme Index. The Lean Index is used for flocks not within a Sire Reference Scheme, and a Scheme Index is used for flocks within a Sire Reference Scheme (this is a co-operative breeding scheme, which link flocks by using a team of elite rams). The main difference between the two Indices is that the Lean Index cannot be compared across farms, as the score is determined specifically to the environment present on that farm, whereas the Scheme Index can be compared across farms within the same Sire Reference Scheme. In both cases, the EBVs cannot be compared across breeds.

6.2.2 Analysis of data

Signet holds the production indices and EBVs for around 700 flocks, of which seven farms are already part of the IAH field-based scrapie study (described in Chapter 2, page 23), and which have agreed to allow access to their EBV data to use in an analysis of the relationship between *PrP* genotype and productivity. Table 6.1 provides a summary of the breeds, number of sheep and scrapie status of each of the seven farms involved in this study.

Table 6.1 Summary information on the farms involved in this study

Farm ID	Scrapie	Breed	Approx. no of Sheep
D34	No	Poll Dorset	550
H19	No	Poll Dorset	480
P27	Yes	Texel	225
B11	Yes	Texel	245
	Yes	Suffolk	225
S03	Yes	Charollais	215
T49	No	Charollais	105
L47	No	Welsh Mountain	260

Each of the seven farms were initially analysed separately, and then six were analysed as pairs as they were within the same Sire Reference Schemes. As these schemes involve the use of a common pool of rams, EBVs and Index scores can be compared across the paired flocks. The pairings for analysis were as follows: Farms H19 and D34 (two scrapie-free farms) were designated Pair 1; B11 (Texels) and P27 (two scrapie-affected farms) were designated Pair 2; and S03 and T49 (one scrapie-affected, one scrapie-free farm) were designated Pair 3.

The response variables were the values provided by Signet, the original phenotypic measurements (weights, muscle and fat depths) and calculated EBVs (table 6.2).

Table 6.2 Traits measured by Signet analysed in this study (Meat and Livestock Commission, 2005)

Trait	Definition
8-week weight	Weight of lambs in kg at around 8 weeks of age
Scan weight	Weight of lambs in kg at around 20-21 weeks of age
Muscle depth	Muscle depth in mm measured over 3rd lumbar vertebrae by ultrasonic scanning at 20/21 weeks
Fat depth	Fat depth in mm measured over 3rd lumbar vertebrae by ultrasonic scanning at 20/21 weeks
8-week weight EBV	Selection for higher 8 Week EBV will result in heavier lambs at 8 weeks of age.
Scan weight EBV	Selection for higher Scan Weight EBV will result in heavier lambs at 20-21 weeks of age.
Muscle depth EBV	Selection for higher Muscle Depth EBVs will increase lamb muscularity and hence the lean meat content of the carcase.
Fat depth EBV	Selection for lower Fat Depths EBVs will result in less fat in the carcase.
Maternal ability EBV	The higher the Maternal Ability EBV, the better ewe lambs will perform as mothers (e.g. milking ability).
Mature size EBV	Selection for a higher Mature Size EBV will increase mature size
Litter size EBV	Selection for larger Litter Size EBV will increase litter size.
Index	This is an overall score calculated for each animal by combining the EBVs for Scan Weight, Muscle Depth and Fat Depth EBVs.
Lean conformation EBV	Selection for higher Lean Conformation EBVs will increase the lean meat content of the carcase.
Fat weight EBV	Selection for lower values will decrease the amount of fat in the carcase of a lamb
Muscularity EBV	Selection for higher Muscularity EBVs will select for lambs with greater gigot muscle size
FEC / FEC 2 EBV	Selection for lower FEC (Faecal Egg Count), will select for sheep with increased resistance to intestinal parasites

Scan weight was analysed as a growth rate as the exact age at which the scan weight was recorded is available. This growth rate was calculated by dividing the weight by the age in days. Explanatory variables were Farm, year of birth (YoB); Sex; genotype group (as defined in Chapter 2) and Season (whether born 'early' in the year: January to June or 'late': July – December. Season was not included in the analysis of the

EBVs. Each of these explanatory variables was treated as fixed effects, and ANOVA carried out (using S-Plus©) on each response variable to determine which of the fixed effects had a significant effect on the productivity parameters.

A maximal model of main factors was initially fitted in each case and then reduced to the simplest models, with all terms significant. Additionally, for the dataset Pair 3, the interaction between genotype group and the farms' scrapie status were also included in the model to investigate if the performance of susceptible sheep of the same breed would differ between scrapie-affected and scrapie-unaffected farms. When assessing the effects of each of the main factors and interactions, a 1% significance level was used to reduce the number of type I errors as per Chapter 3.

Once a minimal model was determined, 1-way ANOVA and pair-wise comparison techniques (Fisher Least Significant Differences method at the 5% level) were used to determine which susceptibility levels were significantly different to each other. The fitted values from the model were designated the response variables, and susceptibility grouping designated the explanatory variable. These fitted values were also used to generate graphs of the mean and 95% confidence limits for each productivity trait found to have a significant relationship with genotype.

6.3 Results

6.3.1 Data sources

The data Signet provided was a combination of phenotypic measurements and EBVs (table 6.3), which were used to investigate potential relationships between *PrP* genotype and productivity traits on each farm.

Table 6.3 The Signet data available for each farm

	Original Measurements				EBVs				
Farm	8-week weight	Scan weight	Muscle depth	Fat depth	8-week weight	Scan weight	Muscle depth	Fat depth	Index
D34	√	√	√	√	√	√	√	√	√
H19	√	√	√	√	√	√	√	√	√
P27	√	√	√	√	√	√	√	√	√
B11 Texel	-	-	-	-	√	√	√	√	√
B11 Suffolk	-	-	-	-	√	√	√	√	√
S03	√	√	√	√	√	√	√	√	√
T49	√	√	√	√	√	√	√	√	√
L47	√	√	√	√	√	√	√	√	√

	EBVs							
Farm	Maternal ability	Litter size	Mature size	Lean conformation	Fat weight	Muscularity	FEC	FEC2
D34	√	√	√	-	-	-	-	-
H19	√	√	√	-	-	-	-	-
P27	√	√	√	√	√	√	√	√
B11 Texel	-	-	-	-	-	-	-	-
B11 Suffolk	-	-	-	-	-	-	-	-
S03	√	√	√	√	√	√	√	√
T49	√	√	√	√	√	√	√	√
L47	√	√	√	-	-	-	-	-

√ – Indicates which Signet measurements are available for that farm

FEC / FEC2 – Faecal Egg Count for two different types of worm

Phenotypic measurements were available for Farm L47 but not included in the analysis as the data only represented 10 animals.

6.3.2 Initial analysis

A summary of the analytical results is shown in tables 6.4 – 6.6 defined by genotype group as follows: Risk group (6.4); Allelic group (6.5) and ERA group (6.6). Traits with a significant association with genotype group at the 1% level are highlighted in bold. Appendix 3 (page 215), section 3.1 contains the graphical representation of the results in these tables, and section 3.2 highlights the quantitative variations in the mean values for each of the genotype groups in these graphs. Appendix 4 contains the ANOVA tables of results for these analyses. Scan weight; Muscle depth; Fat Depth; Fat Depth EBV; Muscularity EBV; FEC and FEC2 EBV did not have any significant relations with genotypes at the 1% level.

Tables 6.4 – 6.6 Associations between Signet parameters and genotype grouping. 'ns' – not significant, p >0.05; 'n/a' – not applicable and individual farms with 'sc' after the farm codes are scrapie affected. The differences between the mean performances of the genotype groups are also presented.

Table 6.4 Associations between Signet parameters and Risk group on (a) Individual Farms and (b) Paired farms. Graphical representations of results significant at the 1% level are shown in Appendix 3, section 3.1, figures 1i – xx.

6.4a

	8-week weight	Scan weight	Muscle depth	Fat depth
Farm	p-value	p-value	p-value	p-value
D34	<0.05	ns	ns	ns
H19	<0.05	<0.05	ns	ns
P27 (sc)	<0.05	<0.05	ns	ns
B11 Texel (sc)	n/a	n/a	n/a	n/a
S03 (sc)	<0.05	<0.05	ns	ns
T49	ns	ns	ns	ns
B11 Suffolk (sc)	n/a	n/a	n/a	n/a
L47	n/a	n/a	n/a	n/a

	8-week weight EBV			Scan weight EBV		
	p-value	Figure	Summary	p-value	Figure	Summary
D34	**0.005**	**i**	**R1>R3,R5>R4**	**<0.001**	**iv**	**R1,R3,R5>R4**
H19	**0.009**	**ii**	**R5>R3,R4>R1**	<0.05		
P27 (sc)	ns	-	-	ns	-	-
B11 Texel (sc)	**0.007**	**Iii**	**R1,R2,R3>R4**	<0.05	-	-
S03 (sc)	ns	-	-	ns	-	-
T49	ns	-	-	ns	-	-
B11 Suffolk (sc)	ns	-	-	ns	-	-
L47	ns	-	-	ns	-	-

Table 6.4a cont.

	Muscle depth EBV			Fat depth EBV
	p-value	Figure	Summary	p-value
D34	**<0.001**	**v**	**R1>R3>R4,R5**	ns
H19	<0.05	-	-	ns
P27 (sc)	ns	-	-	ns
B11 Texel (sc)	ns	-	-	ns
S03 (sc)	ns	-	-	ns
T49	ns	-	-	ns
B11 Suffolk (sc)	ns	-	-	ns
L47	ns	-	-	ns

	Maternal ability EBV			Mature size EBV		
	p-value	Figure	Summary	p-value	Figure	Summary
D34	**<0.001**	**vi**	**R1,R3,R5>R4**	**0.001**	**ix**	**R1,R3>R5>R4**
H19	**0.005**	**vii**	**R5>R3,R4>R1**	ns	-	-
P27 (sc)	ns	-	-	ns	-	-
B11 Texel (sc)	n/a	-	-	n/a	-	-
S03 (sc)	**0.001**	**viii**	**R1>R3>R4,R5**	ns	-	-
T49	ns	-	-	ns	-	-
B11 Suffolk (sc)	n/a	-	-	n/a	-	-
L47	ns	-	-	ns	-	-

	Litter size EBV			Index		
	p-value	Figure	Summary	p-value	Figure	Summary
D34	**<0.001**	**x**	**R1,R3>R4,R5**	**0.002**	**xi**	**R1,R3>R4,R5**
H19	ns	-	-	ns	-	-
P27 (sc)	ns	-	-	ns	-	-
B11 Texel (sc)	n/a	-	-	<0.05	-	-
S03 (sc)	ns	-	-	ns	-	-
T49	ns	-	-	ns	-	-
B11 Suffolk (sc)	n/a	-	-	ns	-	-
L47	ns	-	-	ns	-	-

	Lean conformation EBV	Muscularity EBV	FEC EBV	FEC2 EBV
	p-value	p-value	p-value	p-value
D34	n/a	n/a	n/a	n/a
H19	n/a	n/a	n/a	n/a
P27 (sc)	ns	<0.05	ns	ns
B11 Texel (sc)	n/a	n/a	n/a	n/a
S03 (sc)	ns	ns	ns	ns
T49	ns	ns	ns	ns
B11 Suffolk (sc)	n/a	n/a	n/a	n/a
L47	n/a	n/a	n/a	n/a

Table 6.4b

	8-week weight			Scan weight	Muscle depth	Fat depth
Pairing	p-value	Figure	Summary	p-value	p-value	p-value
Pair 1	**0.003**	**xii**	**R1,R4<R3<R5**	ns	ns	ns
Pair 2	n/a	-	-	n/a	n/a	n/a
Pair 3	ns	-	-	interaction effects <0.05	ns	ns

	8-week weight EBV			Scan weight EBV		
	p-value	Figure	Summary	p-value	Figure	Summary
Pair 1	**0.007**	**xiii**	**R1,R3,R5>R4**	**0.002**	**xv**	**R5>R1; R1,R3,R5>R4**
Pair 2	<0.05	-	-	<0.05	-	-
Pair 3	**0.006**	**xiv**	**R1>R3,R4,R5;R3>R4**	<0.05	-	-

	Muscle depth EBV			Fat depth EBV	Index
	p-value	Figure	Summary	p-value	p-value
Pair 1	**0.001**	**xvi**	**R1,R5>R3>R4**	ns	ns
Pair 2	**0.002**	**xvii**	**R4>R3>R1>R2**	ns	ns
Pair 3	ns	-	-	ns	<0.05

	Maternal ability EBV			Mature size EBV	Litter size EBV		
	p-value	Figure	Summary	p-value	p-value	Figure	Summary
Pair 1	**0.002**	**xviii**	**R5>R3>R1>R4**	ns	**<0.001**	**xx**	**R1>R3>R4>R5**
Pair 2	n/a	-	-	n/a	n/a	-	-
Pair 3	**0.004**	**xix**	**R1>R3>R4,R5**	<0.05	ns	-	-

	Lean conformation EBV	Muscularity EBV	FEC EBV	FEC2 EBV
	p-value	p-value	p-value	p-value
Pair 1	n/a	n/a	n/a	n/a
Pair 2	n/a	n/a	n/a	n/a
Pair 3	<0.05	ns	ns	ns

Table 6.5 Associations between Signet parameters and Allelic group on (a) Individual Farms and (b) Paired farms. Graphical representations of results significant at the 1% level are shown in Appendix 3, section 3.1, figures 2i – xxi.

6.5a

	8-week weight			Scan weight	Muscle depth	Fat depth
Farm	p-value	Figure	Summary	p-value	p-value	p-value
D34	<0.05	-	-	ns	ns	ns
H19	ns	-	-	ns	<0.05	ns
P27 (sc)	**0.004**	**i**	**I>III**	<0.05	ns	<0.05
B11 Texel (sc)	n/a	-	-	n/a	n/a	n/a
S03 (sc)	ns	-	-	ns	ns	ns
T49	ns	-	-	ns	ns	ns
B11 Suffolk (sc)	n/a	-	-	n/a	n/a	n/a
L47	n/a	-	-	n/a	n/a	n/a

	8-week weight EBV			Scan weight EBV		
	p-value	Figure	Summary	p-value	Figure	Summary
D34	**0.004**	**ii**	**I>IV>II,III**	**<0.001**	**iv**	**I,IV>II;I>III**
H19	<0.05	-	-	**0.008**	**v**	**IV>I,III>II**
P27 (sc)	ns	-	-	ns	-	-
B11 Texel (sc)	**0.006**	**iii**	**I,II>III**	<0.05	-	-
S03 (sc)	ns	-	-	ns	-	-
T49	ns	-	-	ns	-	-
B11 Suffolk (sc)	ns	-	-	ns	-	-
L47	ns	-	-	ns	-	-

	Muscle depth EBV			Fat depth EBV	Maternal ability EBV		
	p-value	Figure	Summary	p-value	p-value	Figure	Summary
D34	**<0.001**	**vi**	**I>II,IV>III**	ns	**<0.001**	**viii**	**I,IV>II,III**
H19	**0.001**	**vii**	**II,IV>I>III**	ns	**0.004**	**ix**	**IV>III>I,II**
P27 (sc)	ns	-	-	ns	ns	-	-
B11 Texel (sc)	ns	-	-	<0.05	n/a	-	-
S03 (sc)	ns	-	-	ns	<0.05	-	-
T49	ns	-	-	ns	ns	-	-
B11 Suffolk (sc)	ns	-	-	ns	n/a	-	-
L47	ns	-	-	ns	ns	-	-

Table 6.5a (cont.)

	Mature size EBV			Litter size EBV		
	p-value	Figure	Summary	p-value	Figure	Summary
D34	**0.001**	**x**	**I>IV>II,III**	**<0.001**	**xi**	**I>II,III,IV**
H19	ns	-	-	<0.05	-	-
P27 (sc)	ns	-	-	ns	-	-
B11 Texel (sc)	n/a	-	-	n/a	-	-
S03 (sc)	ns	-	-	ns	-	-
T49	ns	-	-	ns	-	-
B11 Suffolk (sc)	n/a	-	-	n/a	-	-
L47	ns	-	-	ns	-	-

	Index			Lean conformation EBV	Muscularity EBV	FEC EBV	FEC2 EBV
	p-value	Figure	Summary	p-value	p-value	p-value	p-value
D34	**0.004**	**xii**	**I>II,III,IV**	n/a	n/a	n/a	n/a
H19	ns	-	-	n/a	n/a	n/a	n/a
P27 (sc)	ns	-	-	ns	<0.05	ns	ns
B11 Texel (sc)	<0.05	-	-	n/a	n/a	n/a	n/a
S03 (sc)	ns	-	-	ns	ns	ns	ns
T49	ns	-	-	ns	ns	ns	ns
B11 Suffolk (sc)	ns	-	-	n/a	n/a	n/a	n/a
L47	ns	-	-	n/a	n/a	n/a	n/a

Table 6.5b

	8-week weight	Scan weight	Muscle depth	Fat depth
Pairing	p-value	p-value	p-value	p-value
Pair 1	ns	ns	ns	ns
Pair 2	n/a	n/a	n/a	n/a
Pair 3	ns	ns	ns	ns

	8 -week weight EBV			Scan weight EBV		
	p-value	Figure	Summary	p-value	Figure	Summary
Pair 1	**<0.001**	**xiii**	**I,IV>III>II**	**<0.001**	**xvi**	**IV>I,III>II**
Pair 2	**0.008**	**xiv**	**I>III**	<0.05	-	-
Pair 3	**0.007**	**xv**	**I>II,III,IV**	<0.05	-	-

	Muscle depth EBV			Fat depth EBV	Maternal ability EBV		
	p-value	Figure	Summary	p-value	p-value	Figure	Summary
Pair 1	**0.007**	**xvii**	**I,IV>II>III**	ns	**<0.001**	**xviii**	**IV>III>I>II**
Pair 2	ns	-	-	ns	n/a	-	-
Pair 3	ns	-	-	ns	<0.05	-	-

	Mature size EBV			Litter size EBV		
	p-value	Figure	Summary	p-value	Figure	Summary
Pair 1	**<0.001**	**xix**	**III>I>IV>II**	**<0.001**	**xx**	**I>II>IV;I>III**
Pair 2	n/a	-	-	n/a	-	-
Pair 3	<0.05	-	-	ns	-	-

	Index			Lean conformation EBV	Muscularity EBV	FEC EBV	FEC2 EBV
	p-value	Figure	Summary	p-value	p-value	p-value	p-value
Pair 1	**0.001**	**xxi**	**I,IV>II;I>III**	n/a	n/a	n/a	n/a
Pair 2	<0.05	-	-	n/a	n/a	n/a	n/a
Pair 3	<0.05	-	-	<0.05	ns	<0.05	ns

Table 6.6 Associations between Signet parameters and ERA group on (a) Individual Farms and (b) Paired farms. Graphical representations of results significant at the 1% level are shown in Appendix 3, section 3.1, figures 3i – xviii.

6.6a

	8-week weight	Scan weight	Muscle depth	Fat depth
Farm	p-value	p-value	p-value	p-value
D34	ns	ns	ns	ns
H19	<0.05	<0.05	ns	ns
P27 (sc)	<0.05	ns	ns	ns
B11 Texel (sc)	n/a	n/a	n/a	n/a
S03 (sc)	<0.05	<0.05	ns	ns
T49	ns	ns	ns	ns
B11 Suffolk (sc)	n/a	n/a	n/a	n/a
L47	n/a	n/a	n/a	n/a

	8-week weight EBV	Scan weight EBV			Muscle depth EBV			Fat depth EBV
	p-value	p-value	Figure	Summary	p-value	Figure	Summary	p-value
D34	<0.05	**<0.001**	**i**	**a,b>d>c**	**<0.001**	**ii**	**a>b>c,d**	ns
H19	<0.05	<0.05	-	-	ns	-	-	ns
P27 (sc)	ns	ns	-	-	ns	-	-	ns
B11 Texel (sc)	<0.05	<0.05	-	-	ns	-	-	ns
S03 (sc)	ns	ns	-	-	ns	-	-	ns
T49	ns	ns	-	-	ns	-	-	ns
B11 Suffolk (sc)	ns	ns	-	-	ns	-	-	ns
L47	ns	ns	-	-	ns	-	-	ns

	Maternal ability EBV			Mature size EBV			Litter size EBV		
	p-value	Figure	Summary	p-value	Figure	Summary	p-value	Figure	Summary
D34	**<0.001**	**iii**	**a,b,d>c**	**0.001**	**v**	**a,b>d>c**	**<0.001**	**vi**	**a,b>c,d**
H19	<0.05	-	-	<0.05	-	-	<0.05	-	-
P27 (sc)	ns	-	-	ns	-	-	ns	-	-
B11 Texel (sc)	n/a	-	-	n/a	-	-	n/a	-	-
S03 (sc)	**0.001**	**iv**	**a>b>c,d**	ns	-	-	ns	-	-
T49	ns	-	-	ns	-	-	ns	-	-
B11 Suffolk (sc)	n/a	-	-	n/a	-	-	n/a	-	-
L47	ns	-	-	ns	-	-	ns	-	-

Table 6.6a (cont.)

	Index			Lean conformation EBV	Muscularity EBV	FEC EBV	FEC2 EBV
	p-value	Figure	Summary	p-value	p-value	p-value	p-value
D34	**0.003**	**vii**	**a,b>c,d**	n/a	n/a	n/a	n/a
H19	ns	-	-	n/a	n/a	n/a	n/a
P27 (sc)	ns	-	-	ns	<0.05	ns	ns
B11 Texel (sc)	<0.05	-	-	n/a	n/a	n/a	n/a
S03 (sc)	ns	-	-	ns	ns	ns	ns
T49	ns	-	-	ns	ns	ns	ns
B11 Suffolk (sc)	ns	-	-	n/a	n/a	n/a	n/a
L47	ns	-	-	n/a	n/a	n/a	n/a

Table 6.6b

	8-week weight			Scan weight	Muscle depth	Fat depth
Pairing	p-value	Figure	Summary	p-value	p-value	p-value
Pair 1	**0.006**	**viii**	**c<a<b<d**	ns	ns	ns
Pair 2	n/a	-	-	n/a	n/a	n/a
Pair 3	ns	-	-	<0.05	ns	ns

	8-week weight EBV			Scan weight EBV			Muscle depth EBV	Fat depth EBV
	p-value	Figure	Summary	p-value	Figure	Summary	p-value	p-value
Pair 1	**0.001**	**ix**	**a,b,d>c**	**<0.001**	**xi**	**a,b,d>c**	ns	ns
Pair 2	<0.05	-	-	ns	-	-	ns	ns
Pair 3	**0.005**	**x**	**a>b,c,d; b>d**	<0.05	-	-	ns	ns

	Maternal ability EBV			Mature size EBV			Litter size EBV		
	p-value	Figure	Summary	p-value	Figure	Summary	p-value	Figure	Summary
Pair 1	**<0.001**	**xii**	**d>b>a>c**	**<0.001**	**xiv**	**b>d>a>c**	**<0.001**	**xv**	**a>b>c>d**
Pair 2	n/a	-	-	n/a	-	-	n/a	-	-
Pair 3	**0.002**	**xiii**	**a>b,c>d**	<0.05	-	-	ns	-	-

	Index			Lean conformation EBV			Muscularity EBV	FEC EBV	FEC2 EBV
	p-value	Figure	Summary	p-value	Figure	Summary	p-value	p-value	p-value
Pair 1	**0.001**	**xvi**	**a,b>d>c**	n/a	-	-	n/a	n/a	n/a
Pair 2	ns	-	-	n/a	-	-	n/a	n/a	n/a
Pair 3	**0.008**	**xvii**	**a>b,c>d**	**0.009**	**xviii**	**a>b>d; a>c**	ns	<0.05	ns

Table 6.7 is a summary of the number of analyses performed and the number found to have significant relationships at the 1% level. The column headed 'Scrapie-affected' under the 'Number on Paired Farms' heading included the dataset consisting of one scrapie-free and one scrapie-affected farm (Pair 3).

Table 6.7 Summary of the number of analyses performed.

	Total number of relationships		Number on individual farms		Number on paired farms	
	Individual farms	Paired farms	Scrapie-free	Scrapie-affected	Scrapie-free	Scrapie-affected
1% significance level	30	29	25	5	20	9
5% significance level	38	20	17	21	0	20
Not significant	202	50	102	100	16	34
Total	270	99	144	126	36	63

Table 6.8 summarises the effects seen on scrapie-affected and -free farms (including the paired farms), which is based on visual interpretation of trends seen in all the graphs produced. Where an effect is categorised as unclear, the graphs do not lean towards any particular trend.

Table 6.8 Observed effects of increasing susceptibility to scrapie on productivity values on the scrapie-free and scrapie-affected: +ve – scrapie susceptibility is associated with higher productivity values; -ve – resistance to scrapie is associated with higher productivity values; unclear – no trend observed.

	Individual Farms								
	Scrapie-free					Scrapie-affected			
Trait	+ve	-ve	unclear	5%	NS	+ve	-ve	5%	NS
8 Week Weight	-	-	-	4	5	-	1	4	1
Scan Weight	-	-	-	2	7	-	-	4	2
Muscle Depth	-	-	-	1	8	-	-	-	6
Fat Depth	-	-	-	-	9	-	-	1	5
8 Week EBV	1	2	-	3	6	-	2	1	9
Scan EBV	1	3	-	2	6	-	-	3	9
Muscle Depth EBV	1	3	-	1	7	-	-	-	12
Fat Depth EBV	-	-	-	-	12			1	11
Maternal EBV	4	-	1	1	6	-	2	1	3
Mature EBV	-	3	-	1	8	-	-	-	6
Litter Size EBV	-	3	-	2	7	-	-	-	6
Index Score	-	3	-	-	9	-	-	3	9
Lean Conformation EBV	-	-	-	-	3	-	-	-	6
Muscularity EBV	-	-	-	-	3	-	-	3	3
FEC EBV	-	-	-	-	3	-	-		6
FEC 2 EBV	-	-	-	-	3	-	-	-	6
Total	7	17	1	17	102	0	5	21	100

	Paired Farms								
	Scrapie-free					Scrapie-affected			
Trait	+ve	-ve	unclear	5%	NS	+ve	-ve	5%	NS
8 Week Weight	2	-	-	-	1	-	-	-	3
Scan Weight	-	-	-	-	3	-	-	2	1
Muscle Depth	-	-	-	-	3	-	-	-	3
Fat Depth	-	-	-	-	3	-	-	-	3
8 Week EBV	-	1	2	-	-	-	4	2	-
Scan EBV	2	-	1	-	-	-	-	5	1
Muscle Depth EBV	-	-	2	-	1	1	-	-	5
Fat Depth EBV	-	-	-	-	3	-	-	-	6
Maternal EBV	3	-	-	-		-	2	1	-
Mature EBV	-	-	2	-	1	-	-	3	-
Litter Size EBV	-	3	-	-		-	-	-	3
Index Score	-	2	-	-	1	-	1	3	2
Lean Conformation EBV	-	-	-	-	-	-	1	2	-
Muscularity EBV	-	-	-	-	-	-	-	-	3
FEC EBV	-	-	-	-	-	-	-	2	1
FEC 2 EBV	-	-	-	-	-	-	-	-	3
Total	7	6	7	0	16	1	8	20	34

On all individual scrapie-affected farms, there is a negative association between increasing susceptibility and performance. Sheep susceptible to scrapie do not appear to perform as well as their resistant counterparts. This is also the case for the majority of the analyses on the scrapie-free farms: increasing susceptibility is associated with higher productivity values in only seven out of the 25 analyses, with one unclear result. On the paired farms, again on the two scrapie-affected pairings increasing susceptibility is predominately associated with reduced productivity. On the scrapie-free pairings, the results are more variable. Seven of the 20 analyses indicated that increased susceptibility was associated with increased productivity; whereas six analyses indicated an opposite effect and no clear pattern of association can be determined for the rest.

6.3.3 Post-hoc analyses 1 – ARR/VRQ effect

In 35 out of the 59 analyses sheep encoding ARR/VRQ (Risk group 4; Allelic group II or ERA group c) appeared to have low productivity values (table 6.9). This suggests that there may be some cost associated with the genotype ARR/VRQ, which was further investigated by pooling the farm data, and comparing the productivity parameters to the Allelic grouping using ANOVA, while accounting for YoB, Breed and Farm differences. Where genotype was significant at the 5% level the differences in performance of each genotype of sheep were compared to the designated control genotype, ARR/VRQ, using Fisher's Least Significant Differences (LSD) test.

Table 6.9 A summary of which genotype groups have the lowest and highest mean productivity parameters (number of individual farms in brackets).

		Number of analyses	
		lowest	highest
Risk group	R1	3 (2)	9 (5)
	R2	1 (0)	1 (1)
	R3	0	2 (2)
	R4	14 (8)	1 (0)
	R5	2 (1)	7 (3)
Allelic group	I	0	12 (8)
	II	10 (5)	0
	III	9 (6)	1 (0)
	IV	2 (1)	8 (4)
ERA group	a	0	11 (4)
	b	0	5 (3)
	c	11 (5)	0
	d	7 (2)	2 (0)

All the productivity parameters are presented in table 6.10, along with which genotypes had higher mean values than ARR/VRQ sheep. The results suggest that ARR/VRQ sheep do not perform as well as other genotypes of sheep, although these sheep have significantly lowest mean values in only two models.

Table 6.10 Genotype classes of sheep with higher mean productivity scores than ARR/VRQ sheep. Values in italics indicate analyses significant at 5% level. Values in bold italic indicate analyses significant at the 1% level

Productivity parameters	Allelic groups of sheep with higher mean values for each Signet productivity parameter than ARR/VRQ (group II) sheep
8-week weight	*I*, III, IV
Scan weight	I, ***IV***
Muscle depth	IV
Fat depth	-
8-week weight EBV	*I*, III, ***IV***
Scan weight EBV	***I, III, IV***
Muscle depth EBV	I, III, *IV*
Fat depth EBV	I, III, *IV*
Mature size EBV	***I, III***, IV
Maternal ability EBV	***I, III, IV***
Litter size EBV	*I*, III
Index	*I*, III, *IV*
Lean conformation EBV	*I*
Fat weight EBV	I, III
Muscularity EBV	-
FEC EBV	III, IV
FEC 2 EBV	I, III

6.3.4 Post-hoc analyses 2 – Sire effects

The differences in the performances of sheep between the farms and the relationships seen may be due to a founder effect of a few rams, so this was evaluated on all the farms with sire data by assessing the number of sires per lamb on each farm. Only six farms had sire data: D34, H19, B11 Texel, T49, S03 and P27. On four of these farms (D34, H19, S03 and P27) each ram sired less than 10% of the flock (except on Farm S03, where one ram out of 43 sired just over 10% of the sheep involved in the analysis – Appendix 5), so a Sire effect was unlikely.

There were no relationships with genotype on farm T49 so ram effect was not investigated. B11 Texel was investigated for sire effects on the relationships between

susceptibility groupings (Risk group and Allelic group) and *8- week weight EBV*, after accounting for YoB, using mixed models with 'Sire' as a random effect. The results of these analyses are presented in table 6.11.

Table 6.11 A comparison of the two analytical summaries of linear and mixed models for associations present between *8-week weight EBV* and genotype groupings (Risk group and Allelic group) after accounting for YoB on Farm B11 Texel. The differences in statistical significance of genotype are highlighted in bold.

	Linear models				Mixed models			
	Coef.	s.e.	t-value	Pr(>|t|)	Coef.	s.e.	t-value	p-value
(Intercept)	1.13	0.55	2.05	0.044	1.06	0.67	1.58	0.119
YoB 1999	1.43	0.60	2.39	0.020	1.17	0.72	1.62	0.111
YoB 2000	1.35	0.57	2.39	0.020	1.32	0.70	1.87	0.066
YoB 2001	1.92	0.58	3.29	0.002	1.73	0.72	2.40	0.020
YoB 2002	1.78	0.57	3.15	0.002	1.81	0.72	2.53	0.014
Risk group 2	0.13	0.37	0.34	0.734	0.18	0.30	0.61	0.543
Risk group 3	-0.09	0.23	-0.39	0.699	0.05	0.18	0.29	0.770
Risk group 4	**-1.00**	**0.32**	**-3.14**	**0.002**	**-0.52**	**0.30**	**-1.75**	**0.086**
	Coef.	s.e.	t-value	Pr(>|t|)	Coef.	s.e.	t-value	p-value
(Intercept)	1.09	0.54	2.00	0.049	1.09	0.67	1.61	0.112
YoB 1999	1.46	0.60	2.42	0.018	1.25	0.74	1.70	0.094
YoB 2000	1.31	0.56	2.31	0.024	1.35	0.71	1.89	0.063
YoB 2001	1.91	0.58	3.27	0.002	1.78	0.73	2.44	0.017
YoB 2002	1.82	0.57	3.20	0.002	1.89	0.73	2.60	0.012
II	0.27	0.78	0.34	0.733	-0.03	0.64	-0.04	0.969
III	**-0.82**	**0.25**	**-3.23**	**0.002**	**-0.56**	**0.25**	**-2.28**	**0.026**

In both methods of analysis, *8-week weight EBV* increases with YoB. However, whereas the straightforward linear analysis showed that sheep in Risk group 4 (and Allelic group III) had a significantly lower mean *8-week weight EBV* than sheep in Risk group 1 (and in other Risk groups as shown graphically in Appendix 3, section 3.1, figure 1iii; and lower than Allelic group I, figure 2iii), when the sire effect was taken into account, the significance of the two analyses was reduced to above the 1% threshold (as highlighted in bold in table 6.11), and suggests that the effect seen on Farm B11 Texel is strongly influenced by the rams used, and that there are no

significant differences in performance between sheep in different genotype groups (at the 1% level).

6.3.5 Other associations present

There was an effect of YoB on the Signet parameters, in 54 of the 59 analyses significant at the 1% level (27/30 on individual farms and 27/29 on paired farms, Appendix 4). The aim of the Signet breeding program is to increase the overall EBV and Index scores of the flocks involved, by selective breeding for higher breeding values, and of these 54 analyses, only in two was the mean productivity value observed to decrease as the year of birth increases. These exceptions were *8-week weight* on P27, and *Scan weight EBV* on dataset Pair 2.

Differences in areas of performance between sheep on the farms in each of the paired datasets were highlighted in 10 of the analyses (Appendix 4) and these are summarised in table 6.12. These are most likely reflecting farms differences in, for example, areas such as management or environment. However, as they were significantly influencing the performance of the sheep in these models, the term Farm was included to account for this.

Table 6.12 Differences in overall performance between sheep in the paired datasets. (sc) after the farm indicated that it is a scrapie-affected farm

Trait	Difference between farms
8 week weight	H19 > D34
8 week weight EBV	P27 (sc) > B11 Texel (sc)
Scan weight EBV	D34 > H19
Muscle depth EBV	D34 > H19
Maternal EBV	T49 > S03 (sc)
Mature size EBV	H19 > D34

6.4 Discussion

6.4.1 Initial analysis

Three hundred and sixty-nine analyses were performed to investigate potential relationships between scrapie susceptibility and productivity, 270 on individual farms and 99 on the paired farms. Of these, 30 out of the 270 and 29 of the 99 indicated a significant relationship between productivity and genotype at the 1% level.

On the scrapie-affected farms (including the dataset Pair 3), there is a clear negative effect of susceptibility to scrapie on all productivity traits, except for Muscle depth EBV, which increased as Risk group increased for sheep in the dataset Pair 2. Otherwise, encoding for resistance, even if only in the form of at least one ARR allele, is positively associated with higher productivity. Although it is not expected that a disease status should have an effect on the genetic merit of an animal, in this case as the EBVs are derived from phenotypic performance parameters (for example, weight, muscle depth, fat depth, number of lambs born and successfully raised) of the sheep and its relatives, and so anything which affects these traits, such as chronic disease, will also affect the EBVs to some extent.

The results suggest that on scrapie-affected farms, the performance of susceptible sheep is reduced, possibly due to preclinical disease. Preclinical scrapie will not affect the *8-week weight* parameter directly as there is relatively little maternal transmission, but this weight could also be a reflection of maternal ability. If the dam

is suffering from preclinical disease, then she may not be able to mother her lambs as effectively, contributing to lambs of slightly poorer weight.

Of the 25 relationships detected on individual scrapie-free farms, 17 suggested that resistant sheep were more productive; seven that susceptible sheep were more productive; and one which did not show a clear trend either way, with both susceptible and resistant sheep performing similarly. In this category, although there were significant differences in performance between the genotypes, they could not be linked to increasing or decreasing susceptibility. This is a reflection of differences between farms (these results represented two farms only) and from these results one cannot clearly conclude what association exists between scrapie susceptibility and productivity. On the scrapie-free pairing, the results were more variable, with six analyses suggesting that resistant sheep were more productive; seven that susceptible sheep were more productive; and seven which did not show a clear trend either way.

Chase-Topping *et al.* (2005) investigated the effects of genotype on the reproductive parameters of sheep in scrapie-affected flocks. Their study determined that the overall lifetime breeding success was lower in susceptible sheep as a result of a reduced life span, but did not find any direct association between susceptibility and litter size in scrapie-uninfected sheep on scrapie farms. In this study, the analyses on *Litter size EBV* revealed a strong negative association between increasing susceptibility and EBV. These results suggest that scrapie-susceptible sheep which have not been exposed to disease are more likely to have smaller litter sizes than

more resistant sheep. These studies both indicate that reproductive success is reduced in scrapie-susceptible sheep, both in the absence and the presence of disease.

6.4.2 ARR/VRQ effect

Sheep in the genotype groups Allelic group II, ERA group c and Risk group 4 (i.e. ARR/VRQ animals) appear to have low productivity, as in 35 out of the 49 analyses significant at the 1% level, these sheep had the lowest mean values. Further analysis to investigate this effect involved just the Allelic grouping, and comparison of the performance of all other sheep to ARR/VRQ sheep. The results, although not always statistically significant, show that this genotype had the lowest score in nine out of the 17 productivity parameters assessed. The genotype ARR/VRQ does appear to have some negative effect on fitness, which is not due to the effect of scrapie, as the genotype ARR/VRQ is fairly resistant to scrapie, and this effect is apparent on both scrapie-free and scrapie-affected farms. To assess whether this phenomenon is a chance observation will require further study, to ensure that it is not just confined to this dataset.

6.4.3 Other associations present

It is expected that the weights of lambs and EBVs of the flock should increase over time. This is reflects that with each passing year, there is selection for stock higher genetic merit, so that the overall productivity of the flock improves over time. This was the case in the majority of the analyses where year of birth was significant. The two farms on which there was a reduction in improvement over time were both

scrapie-affected, but there is not enough evidence to determine whether or not this was causal factor.

6.4.4 Economical (biological) vs. statistical significance

The results have highlighted a number of statistically significant differences in performance between genotypes (presented in Appendix 3), although these differences may not be of economical significance to a farmer, and thus be unlikely to have influenced selection for certain genotypes.

The mean values for the phenotypical trait, *8 week weight,* show very small differences between the genotypes, which are likely to be economically insignificant. Between the different genotypes, *8 week weight*, weight varies by up to 1.4kg, with an overall mean roughly around 20kg. In this study, the differences in weight between sheep of different genotypes are unlikely to picked up on visual inspection alone, so farmers could not possibly determine that certain genotypes of sheep grow faster than others. The weight of sheep of different genotypes is relatively similar at this age and also at scan age (20 – 21 weeks).

However, the situation is slightly different for the EBVs. The differences between the genotype groups are more significant economically. For example, the differences in mean *Maternal ability EBV* between R1 and R5 sheep on farm H19 is 0.302: on average R1 sheep have 40% of the EBV of R5 sheep. This difference is more likely to be of interest to the farmer, and would be more likely to influence the farmer's decisions about culling their sheep. This is just one example of the relative

differences between the genotypes of the many presented in Appendix 3, section 3.2. In summary, the results of this study are likely to be of economic importance to the farmer, because of the relatively large differences in genetic merit between the genotypes of sheep.

6.4.5 Conclusion

In summary, the study has shown that on scrapie affected farms the productivity of susceptible animals is lower, which may be due to preclinical infection, as already explained. However, in the absence of disease, it there is no clear association between scrapie susceptibility and the Signet data, as the relationships seen appear to be specific to individual farms.

Some farmers believe that breeding for resistance will result in less productive animals, a belief which is based on past anecdotal evidence. This may be true and, on some farms, susceptible sheep are more productive, but this association was limited to those farms, and may not be true for the entire sheep population. Additionally, the results of this study indicate that the 'superiority' of certain genotypes of sheep appears to be confined the genetic merit of those sheep, with very little phenotypical variation being present, that is observable. These factors may explain why there is little evidence for the superiority of susceptible sheep (Prokopová *et al.*, 2002, de Vries *et al.*, 2004b, de Vries *et al.*, 2004a, Chase-Topping *et al.*, 2005).

7 Modelling how effective various breeding strategies are at reducing the risk of scrapie in individual sheep flocks

7.1 Introduction

The National Scrapie Plan for Great Britain (NSP, see Chapter 1, for more details) aims to eliminate scrapie and other TSEs by breeding sheep genetically resistant to these diseases, and increasing their frequency within UK sheep flocks. This process has been relatively slow, and is complicated by the complex structure of the UK sheep industry, the limited numbers of resistant genotypes in some sheep breeds (Eglin *et al.*, 2005; Townsend *et al.*, 2005), and the fact that the breeding schemes to date have not involved crossbred sheep (DEFRA, 2005b).

It is possible, however, that the alleles associated with scrapie susceptibility will not need to be eliminated completely from the population in order to eradicate the disease. If there are enough animals of resistant genotypes present within a flock, then scrapie will not be able to persist. This is a similar principle to that of 'herd immunity' in a vaccinated population (Anderson and May, 1985). If enough of the population is immune (i.e. resistant to scrapie), then any infected individuals are unlikely to spread the disease; also, any animals susceptible to scrapie are unlikely to come into contact with an infectious individual and, hence, acquire infection (assuming that scrapie-resistant sheep are not carriers). Conversely, if the number of susceptible sheep within a population is high enough, an outbreak may occur if scrapie is introduced to the population.

Kermack and McKendrick developed a model for a disease epidemic within a population in 1927. This model is the *S(L)IR* model, where the population is divided into the groups: *S*usceptibles, (*L*atents, especially in infectious diseases such as scrapie, where there is an incubation period during which the sheep is infected but not yet infective), *I*nfectives and *R*ecovered (in the case of scrapie, *R* becomes *R*emoved as all *I*nfecteds die.) The numbers in each group are not fixed, but vary with time, depending on the infection and recovery (removal) rates (Kermack and McKendrick, 1927; Anderson and May, 1991; Brown and Rothery, 1994). These recovery and removal rates can be used to determine the potential for an epidemic. For an epidemic to occur, the infection rate of susceptible animals must exceed the removal rate of infected animals, and if the number of susceptible animals is less than the relative removal rate (the ratio of the removal rate to the infection rate), then an epidemic cannot occur following the introduction of infection (Kermack and McKendrick, 1927). This is known as the 'threshold theorem' for epidemics. The threshold theorem can also be defined by the term 'basic reproductive rate or ratio' (R_0), and when R_0 is greater than one an epidemic can occur (Anderson and May, 1986; Anderson and May, 1991; Brown and Rothery, 1994). In this chapter R_0 was defined as the number of secondary infections which result from a primary infected individual. For scrapie, the basic reproductive ratio is determined by the genotype frequencies in the flock and the relative susceptibility of different ages and genotypes (Matthews *et al.*, 1999; Hagenaars *et al.*, 2003).

This chapter aims to determine the impact of different breeding strategies on the basic reproductive ratio. In particular, it addresses whether or not the strategies are able to reduce R_0 to below one and, if they are, the number of years required to do so.

7.2 Methods

7.2.1 Modelling flock information

The models used in this chapter were based on those developed by Lewis and Simm (2000), and reflect sheep management practices in the UK. The models were run by Dr. Nicola Man at the Scottish Agricultural College, where there has been a project investigating the effects on genetic merit of varying breeding programmes designed to modify the frequencies of *PrP* genotypes (Man *et al.*, 2006). These models were based on sets of 15 or 17 simulated flocks, the genotypes of which were randomly generated based on the known allele distribution of the breed (Eglin *et al.*, 2005). For this chapter, three breeds were considered: Charollais, Swaledale and Texel, each with different allele frequencies (table 7.1).

Table 7.1 The average allele frequencies of the sheep breeds involved in this chapter: Charollais, Texel and Swaledale (taken from Eglin *et al.*, 2005)

Breed	% ARR	% AHQ	% ARH	% ARQ	% VRQ
Charollais	60.4	0.1	0.2	35.1	4.2
Swaledale	41.1	16.3	0	36.9	5.6
Texel	33.4	4.2	43.7	15.3	3.4

Fifteen flocks of Texel sheep and 15 flocks of Charollais sheep were modelled, with the flocks (initially) ranging in size from 40 to 140 breeding ewes (table 7.2) and

with a lambing percentage of 149%. Thirty ewes in each flock were mated to reference sires (sires used across flock) at a ratio of one sire to 10 ewes, and the rest were mated to rams from within the flock at a ratio of one ram to twenty ewes. The reference sires were chosen with the aim of improving lean growth rates, and a heritability of 0.25 for this trait was assumed (Man *et al.*, 2006).

Table 7.2 Summary of flock sizes, the number of breeding ewes and the total number of sheep including lambs used in the model simulations.

	Number of ewes	
Flock	Charollais/Texel	Swaledale
1	40	100
2	40	150
3	40	200
4	40	250
5	50	300
6	50	300
7	50	350
8	60	350
9	60	400
10	70	450
11	80	450
12	90	500
13	100	500
14	120	550
15	140	600
16	-	650
17	-	700

As the Swaledale is a hill breed, and not a terminal sire breed, the flock structure was slightly different. Seventeen of these flocks were modelled, ranging in size from 100 to 700 breeding ewes (table 7.2). The lambing percentage was lower (127%) and two reference sires per 16 ewes were used in each flock, with the rest of the ewes mated to rams from within the flock, at a ratio of one ram to forty ewes. The reference sires were chosen with the aim of improving lamb weaning weight.

7.2.2 Breeding strategies

In the models, several breeding strategies were considered: these were compared to a 'wild-type' situation in which there was no selection based on PrP genotype, only selection for liveweight or weaning weight. Three selection schemes were investigated. The first two schemes were based on NSP guidelines, and selected against the VRQ allele (i.e. that associated with the highest risk of scrapie) (DEFRA, 2005b). In the first strategy, selection was only applied to rams (strategy 1), while in the second, selection was applied to both ewes and rams (strategy 2). The third strategy was more restrictive and involved sequential selection of rams based on *PrP* genotype. Initially ARR/ARR rams were selected as sires, then, if there were not enough of these rams, ARR-heterozygous (ARR/XXX, but not ARR/VRQ) rams were chosen, followed by XXX/XXX rams (rams not encoding either the ARR or VRQ allele), ARR/VRQ rams, VRQ/XXX rams and VRQ/VRQ rams, in order of preference. This is the most extreme breeding programme of the three (table 7.3). In the simulations, the numbers were such that only the first two genotype groupings were used (ARR/ARR and ARR/XXX).

Table 7.3 Breeding strategies using the model simulations.

Breeding strategy	Selection practice
1	Rams carrying the VRQ allele not used in the flock
2	Ewes and rams carrying the VRQ allele not used in the flock
3	Sequential selection of rams based on genotype, ARR/ARR preferred

7.2.3 Age- and genotype-dependant susceptibilities to scrapie

Age-dependent susceptibilities to scrapie were as determined by St. Rose *et al.* (2006); this work found that susceptibility to scrapie was highest in young sheep

under the age of one year, and lowest in sheep older than two years of age (table 7.4). These values were determined from data on Cheviot sheep managed by the Institute for Animal Health's Neuropathogenesis Unit.

Table 7.4 Age-dependent susceptibilities used in these models. These values were determined in Cheviot sheep by St. Rose *et al.* (2006). LCL – Lower confidence Limit; UCL – Upper confidence Limit

Age / years	Susceptibility	LCL	UCL
0 - 1	0.61	0.48	0.70
1 - 2	0.18	0.08	0.24
> 2	0.03	0.02	0.04

Genotype susceptibilities were derived from studies on the closed Romanov flock kept at Institut National de la Recherche Agronomique, Langlade (INRA). This flock had an outbreak of scrapie in which 304 animals succumbed to the disease between 1993 and 1997; the flock size decreased in that time period from around 600 sheep to about 400 (Hagenaars *et al.*, 2003). The association between the lambing season and scrapie transmission was modelled for this flock (Touzeau *et al.*, 2006), and the genotype susceptibilities from that study were used in this chapter. However, the ARH allele was not represented by the Romanov breed, so relative susceptibilities for genotypes including this allele had to be assumed. This was achieved using data on the estimated number of cases per million sheep by genotype presented in the review by Detwiler and Baylis (2003). The susceptibility selected for each of the ARH/XXX genotypes was that of a genotype with a similar attack rate, and these are presented below in table 7.5 (cf. table 2.1).

Table 7.5 Genotype susceptibilities used in this study. Those susceptibilities highlighted in italics were estimated indirectly, as the ARH allele is not present in the Romanov breed (see main text).

Genotype	Susceptibility
ARR/ARR	0
ARR/ARQ	0.029
ARR/ARH	*0*
ARR/AHQ	0
ARR/VRQ	0.078
ARQ/ARQ	0.403
ARQ/ARH	*0.125*
ARQ/AHQ	0.062
ARQ/VRQ	0.606
ARH/ARH	*0.020*
ARH/AHQ	*0.020*
ARH/VRQ	*0.803*
AHQ/AHQ	0.125
AHQ/VRQ	0.020
VRQ/VRQ	0.803

7.2.4 Calculation of R_o

The basic reproductive number, R_0, was assumed to be given by:

$$R_0 = C\sum_{i=1}^{3}\sum_{j=1}^{15} a_i g_j f_{ij}$$

where C is a scaling constant (see below), a_i is the susceptibility for animals in age class i (table 7.4), g_j is the susceptibility for animals of genotype j (table 7.5) and f_{ij} is the proportion of the flock in age class i of genotype j. The scaling constant, C, was determined so that the expression for R_0 was consistent with previously published studies on the INRA Romanov flock. In particular, R_0 was estimated to be 2.5 for the outbreak (Hagenaars *et al.*, 2003), which in conjunction with the flock's age-genotype profile (Dr. M. Chase-Topping, pers. comm.), was used to determine the value for C in the model, so that it could be applied to other flocks.

Once the scaling factor was determined, the model was used to compute R_0 for each flock in each year of the breeding program based on the genotype frequencies predicted by the gene-flow model (see section 7.2.2). From these results, the time required to drive R_0 below one could be determined. The model was implemented using Fortran, with 100 simulations for each flock. As each simulation produced a flock with a slightly different distribution of genotypes, it was considered that each simulation could be treated as an individual flock, giving a total of 1500 Charollais and Texel flocks, 1700 Swaledale flocks. In each of these models, sheep were randomly mated until year 14, and then on *PrP*- based selection occured from year 15. In the case of the three breeding strategies, this is also the year that the selection for particular genotypes began. Using this information, the fraction of flocks with an R_0 value greater than one for a given simulation in any given year during the breeding programs could also be calculated. This fraction is hereafter denoted by $f(R_0 > 1)$.

7.2.5 Variation in R_0

The above expression assumes that R_0 is the same for all flocks with the identical age and genotype profiles. However, this is unlikely to be the case because of the impact of farm management practices on the risk of scrapie (Hoinville *et al.*, 2000,Gubbins *et al.*, 2006; Sivam *et al.*, 2006). Variation was introduced into the expression for R_0 by using the parameter, w, which takes values drawn from a gamma distribution with mean and variance equal to one. Thus, the new expression for R_0 becomes:

$$R_0 = wC\sum_{i=1}^{3}\sum_{j=1}^{15} a_i g_j f_{ij}$$

where w is a random number drawn from a $\Gamma(1,1)$ distribution. The value of w for each simulation was generated using S-Plus® (see appendix 6).

7.3 Results

7.3.1 Scaling constant

Using the age and genotype susceptibilities presented in Tables 7.4 and 7.5 and the age-genotype profile for the INRA flock, the scaling constant C was calculated to be 46.42 in order to give a value for R_0 of 2.5 (Hagenaars *et al.* 2003).

7.3.2 Effects of the three breeding strategies

Charollais flocks

Although there was a fairly high frequency of the ARR allele (60.4%) in this breed, there were still a high number of flocks with an R_0 greater than one (figure 7.1). The mean R_0 was 1.23 over generations, which may reflect this high proportion of the ARR allele. In the 'wild-type' scenario the fraction of flocks with an R_0 greater than one, $f(R_0 > 1)$, was dependent on the year of the breeding programme. As genetic drift was prevented until year 15, $f(R_0 > 1)$ was fairly constant, but once genetic drift was permitted, $f(R_0 > 1)$ decreased slightly (figure 7.2). This reduction is attributable to slight inbreeding within the flocks (see, e.g. Matthews *et al.* 1999). When any of the three breeding strategies were implemented, $f(R_0 > 1)$ decreased (figure 7.2), although only for the most 'extreme' breeding strategy is the number of flocks with an R_0 greater than one reduced to zero (figure 7.2).

Figure 7.1 The distribution of the R_0 values of the Charollais flocks in year 14. This graph is taken from the simulation in which no selection was applied.

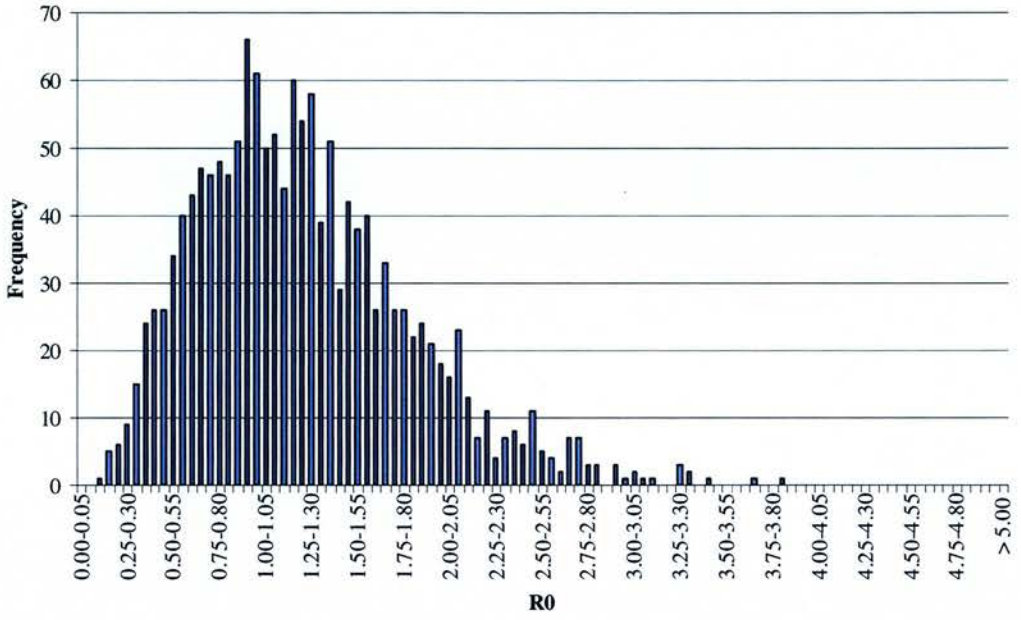
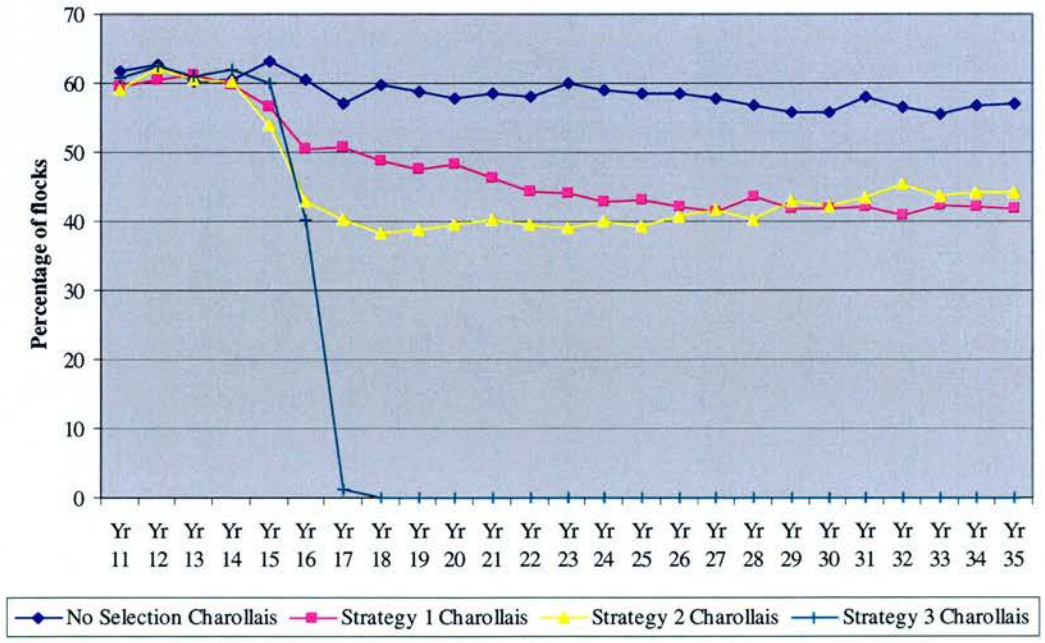


Figure 7.2 The percentages of the 1500 Charollais flocks in each year with an $R_0 > 1$, from year 11 of the simulation, by breeding strategy (with selection being introduced in year 15).



These graphs show there is still a risk of an outbreak of scrapie (if infection were introduced) in a substantial fraction of flocks, even after 20 years of NSP selection as it is currently defined. Breeding strategy 1 resulted in a gradual decline in $f(R_0>1)$, reaching its minimum value of around 40% about 15 years after implementation. Breeding strategy 2 resulted in a similar decline in $f(R_0>1)$, but the minimum value was reached after around three years of selection. Under breeding strategy 3, the number of flocks with an R_0 greater than one was reduced to zero after about three years of selection.

Texel flocks

Although there was a fairly low frequency of the ARR allele (33.4%) in this breed compared to the Charollais breed, the calculated R_0's were relatively low (figure 7.3), with an overall mean of 0.95. This low value may be related to the prevalence of the ARH allele, which, in combination with alleles other than ARQ and VRQ (which occur at low frequencies, table 7.1), is associated with some resistance to scrapie. As for the Charollais flocks, in the 'wild-type' scenario, $f(R_0 > 1)$ decreased slightly once genetic drift was permitted (figure 7.4). Once any of the three breeding strategies were introduced, $f(R_0 > 1)$ decreased, with a decrease to zero only occurring in the most 'extreme' strategy (figure 7.4). The pattern of change in $f(R_0>1)$ under each strategy was similar to that observed for the Charollais breed, although the minimum level for $f(R_0>1)$ (10%) under breeding strategies 1 and 2 was lower for the Texel (figure 7.4; cf. figure 7.2).

Figure 7.3 The distribution of the R_0 values of the Texel flocks in year 14. This graph is taken from the simulation in which no selection was applied.

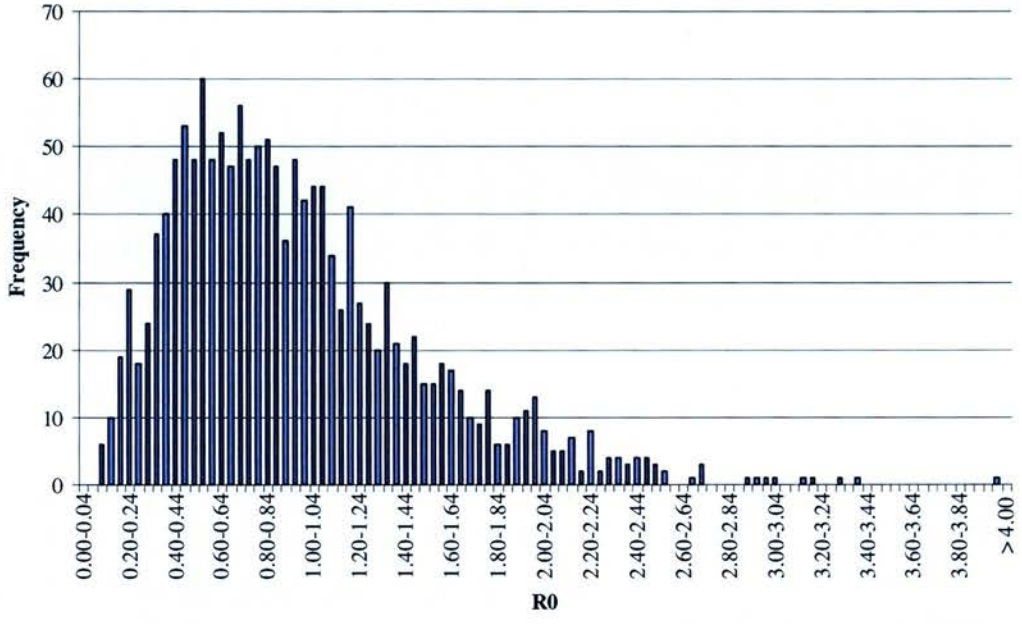
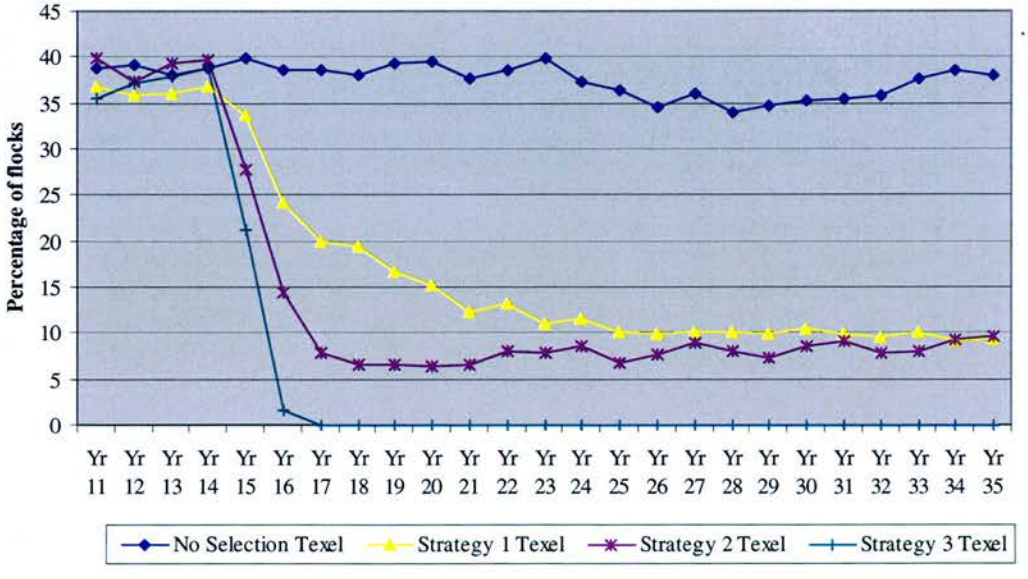


Figure 7.4 The percentages of the 1500 Texel flocks in each year with an $R_0 > 1$, from year 11 of the simulation, by breeding strategy (with selection being introduced in year 15).



Swaledale flocks

The frequency of the ARR allele in this breed was between that of the Charollais and Texel breeds (41.1%) in this breed, and the calculated R_0's were relatively high (figure 7.5). The mean of 1.46 is higher than for both the Texel and Charollais, which is mainly due to the higher frequencies of the ARQ and VRQ alleles in this breed (table 7.1). As for the Charollais and Texel flocks, in the 'wild-type' scenario, $f(R_0 > 1)$ decreased slightly once genetic drift was permitted, although there are still many flocks with high R_0 values (figure 7.6). All three breeding strategies decreased $f(R_0 > 1)$, though it decreased to zero only under the most 'extreme' strategy (figure 7.6). Again, the pattern of change in $f(R_0>1)$ under each strategy was similar to that observed for the Charollais and Texel breed, although the minimum level for $f(R_0>1)$ (60%) under breeding strategies 1 and 2 was much higher for the Swaledale (figure 7.6; cf. figures 7.2 and 7.4).

Figure 7.5 The distribution of the R_0 values of the Swaledale flocks in year 14. This graph is taken from the simulation in which no selection was applied.

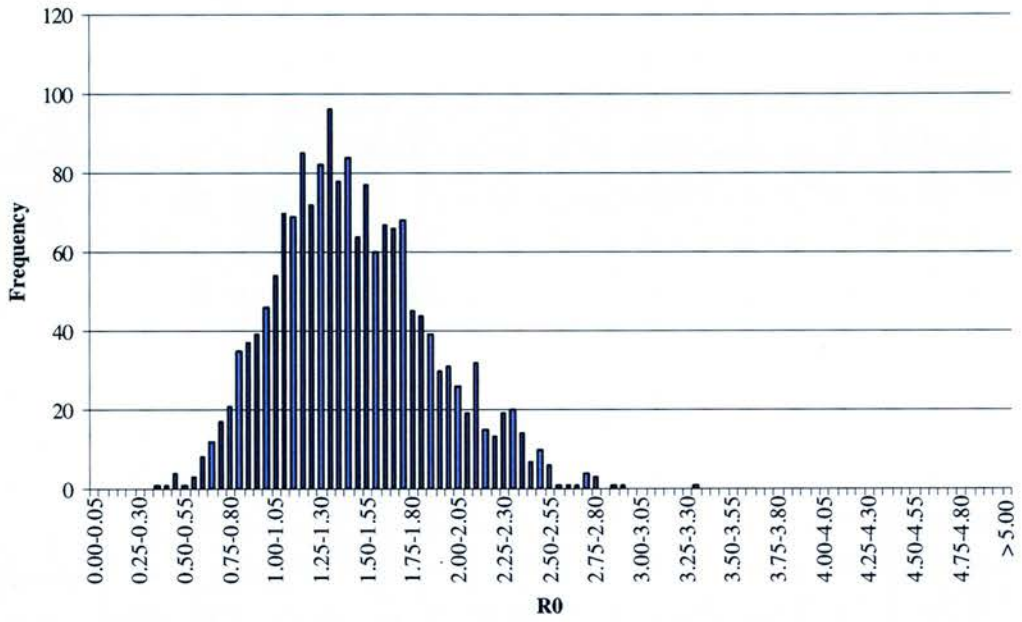
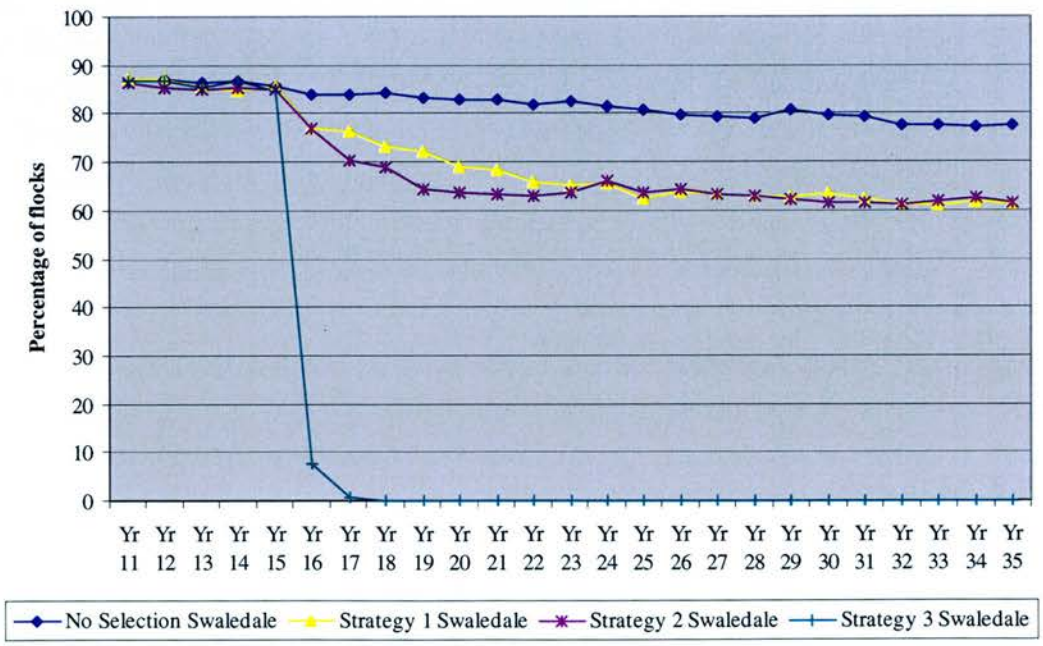


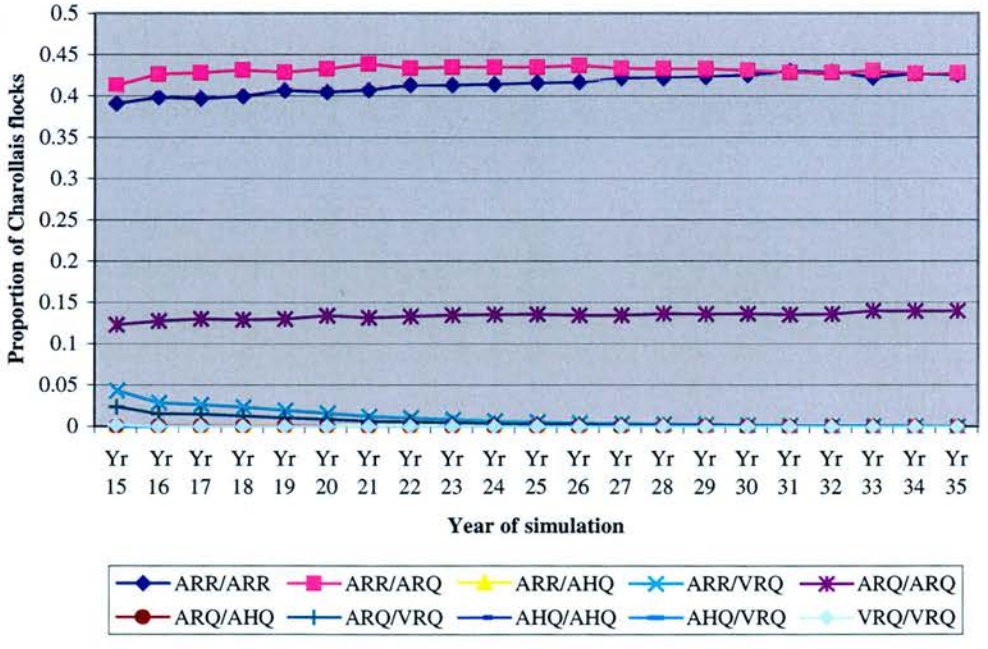
Figure 7.6 The percentages of the 1700 Swaledale flocks in each year with an $R_0 > 1$, from year 11 of the simulation, by breeding strategy (with selection being introduced in year 15).



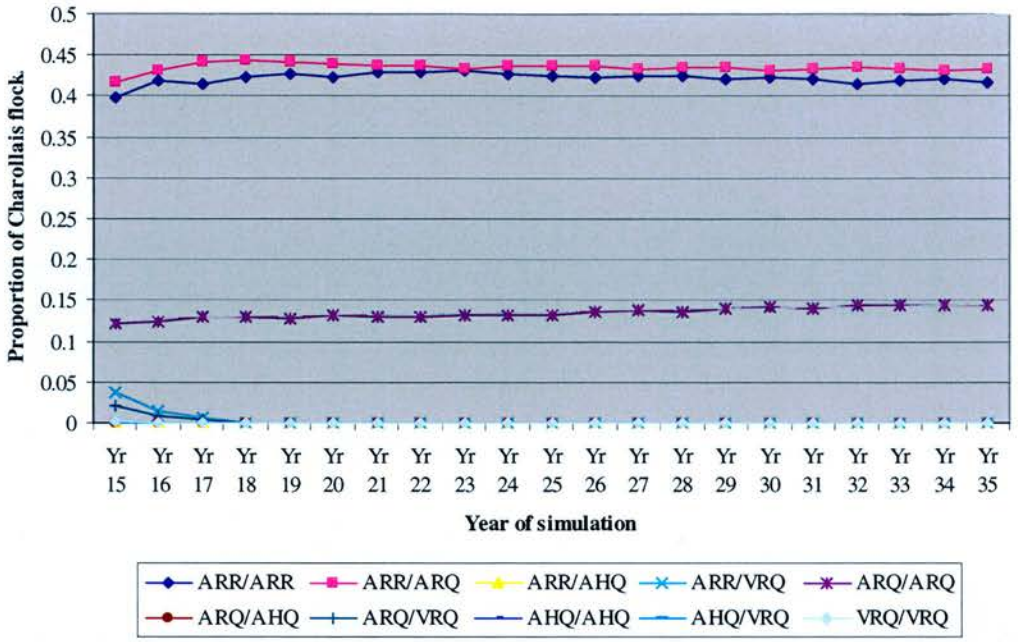
For each of the breeds considered, R_0 did not fall below one in all flocks even after 20 years of NSP-based selection on PrP genotype. This may be explained by inspection of the changes of genotype frequencies as the breeding strategies are applied (figures 7.7, 7.8 and 7.9). Under breeding strategies 1 and 2 those genotypes incorportating the VRQ allele are eliminated from the flock, while the remaining genotypes increase only marginally in frequency. In particular, the frequency of ARR/ARR does not increase markedly in frequency. Consequently, the magnitude of the reduction in R_0 is limited. By contrast, breeding strategy 3 actively selects for ARR/ARR and, hence, the frequency of this genotype does increase markedly for all breeds. Thus, R_0 is substantially reduced in all flocks.

Figure 7.7 Overall proportions of genotypes in the Charollais flocks from year 15 – 35 of the simulations. (a) Breeding strategy 1, (b) Breeding strategy 2 and (c) Breeding strategy 3. Selection begins in year 15.

(a)



(b)


Proportion of Charollais flock
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Yr 15 Yr 16 Yr 17 Yr 18 Yr 19 Yr 20 Yr 21 Yr 22 Yr 23 Yr 24 Yr 25 Yr 26 Yr 27 Yr 28 Yr 29 Yr 30 Yr 31 Yr 32 Yr 33 Yr 34 Yr 35
Year of simulation
ARR/ARR
ARR/ARQ
ARR/AHQ
ARR/VRQ
ARQ/ARQ
ARQ/AHQ
ARQ/VRQ
AHQ/AHQ
AHQ/VRQ
VRQ/VRQ

(c)

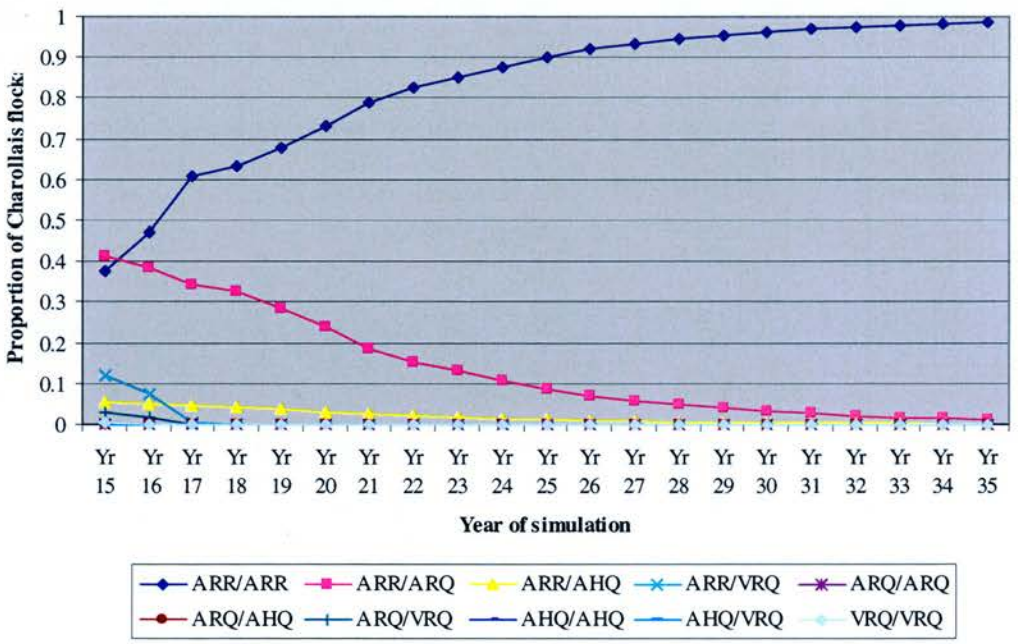
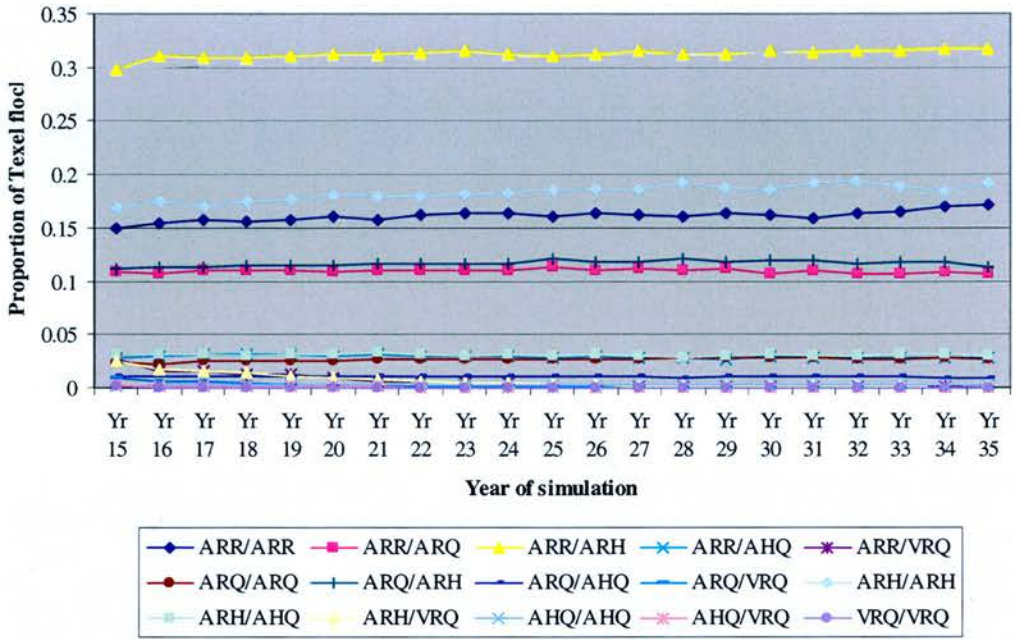

Proportion of Charollais flock
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Yr 15 Yr 16 Yr 17 Yr 18 Yr 19 Yr 20 Yr 21 Yr 22 Yr 23 Yr 24 Yr 25 Yr 26 Yr 27 Yr 28 Yr 29 Yr 30 Yr 31 Yr 32 Yr 33 Yr 34 Yr 35
Year of simulation
ARR/ARR
ARR/ARQ
ARR/AHQ
ARR/VRQ
ARQ/ARQ
ARQ/AHQ
ARQ/VRQ
AHQ/AHQ
AHQ/VRQ
VRQ/VRQ

Figure 7.8 Overall proportions of genotypes in the Texel flocks from year 15 – 35 of the simulations. (a) Breeding strategy 1, (b) Breeding strategy 2 and (c) Breeding strategy 3. Selection begins in year 15.

(a)



(b)

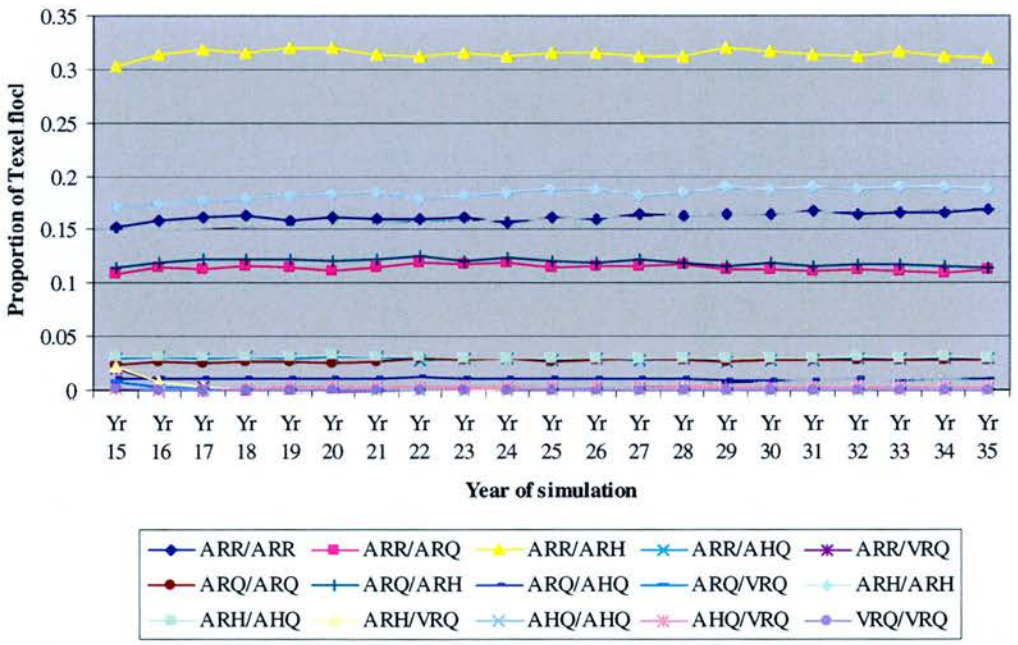


Figure 7.8c

(c)

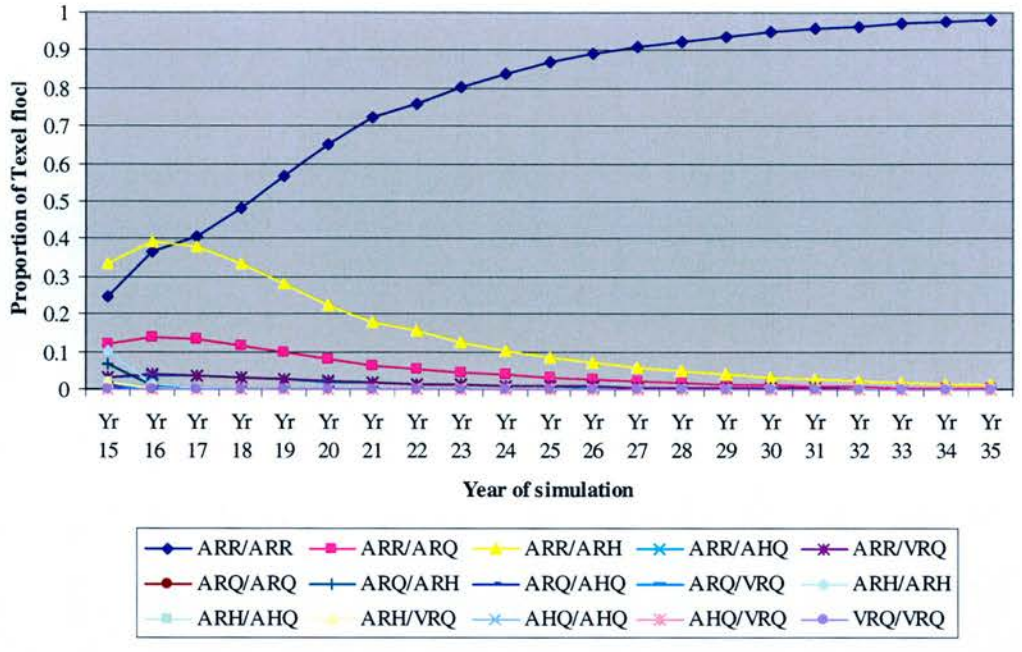


Figure 7.9 Overall proportions of genotypes in the Swaledale flocks from year 15 – 35 of the simulations. (a) Breeding strategy 1, (b) Breeding strategy 2 and (c) Breeding strategy 3. Selection begins in year 15.

(a)

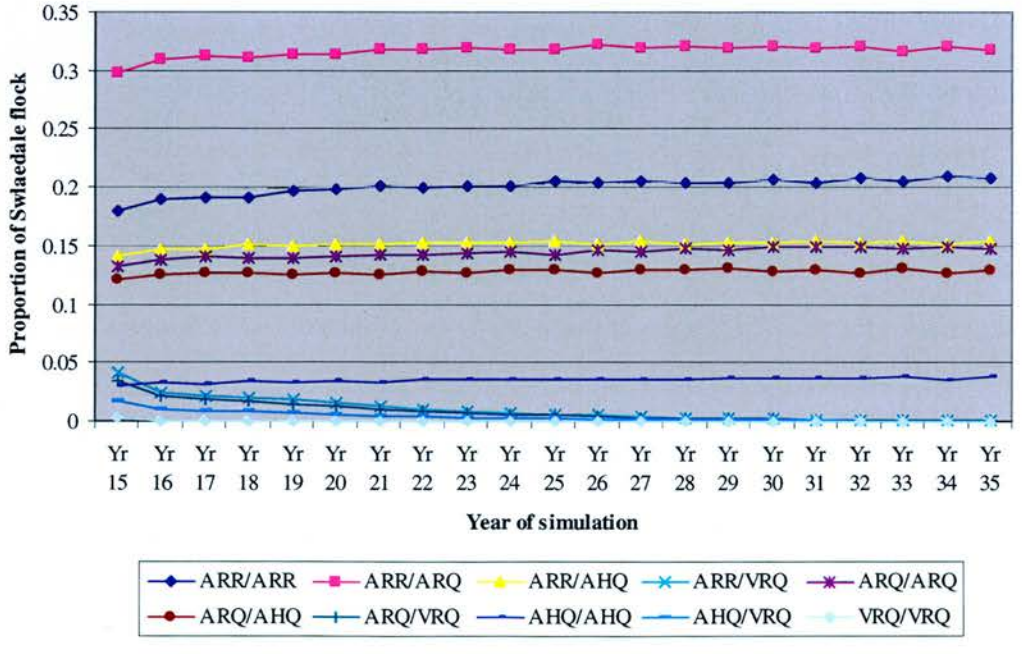
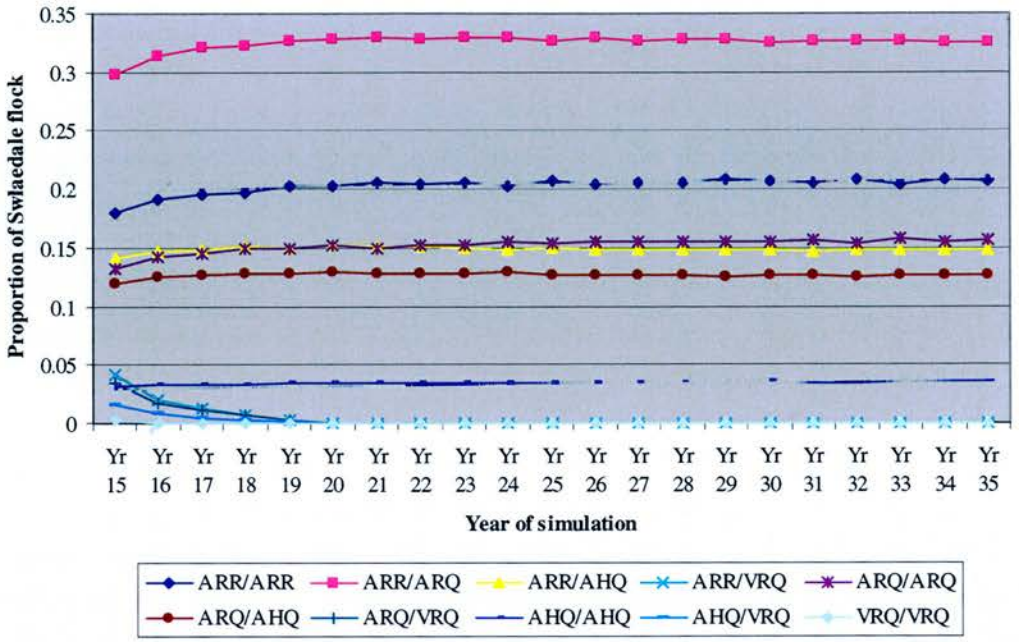
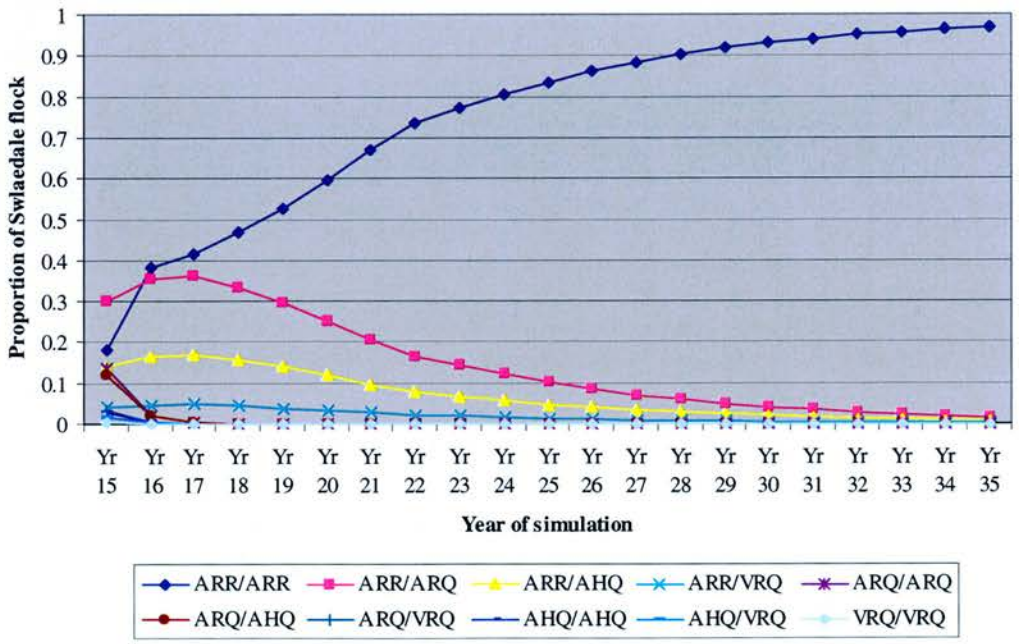


Figure 7.9 cont.

(b)



(c)



7.3.3 Do all alleles associated with susceptibility need to be eliminated in order to reduce R_0 to less than one?

For the three breeding strategies, the associations between genotype profile and R_0 value were generally similar across the breeds. In all breeds, a higher proportion of ARR/ARR sheep was associated with a lower value for R_0, with it falling below one even when only 20% of the flock (depending on the breed) were homozygous for the ARR allele (figure 7.10). The proportion of ARR/ARR sheep did not have to be one (i.e. a completely resistant flock), for R_0 to be less than one, and in the Texel breed, there were instances of R_0 being less than one even when there were no ARR/ARR sheep present (figure 7.10). In all breeds, the relationship between the proportion of sheep encoding VRQ/XXX and R_0 was not particularly strong. However, R_0 could still be greater than one even when the proportion of this genotype was zero (figure 7.11). In the breeding strategies which selected against the VRQ allele (1 and 2), no conclusions could be drawn from the graphs incorporating ARR/XXX for the Texel and Swaledale breeds, although R_0 increased slightly if the proportion of ARR/XXX sheep increased in the Charollais breed. Under the extreme strategy, an increase in R_0 was associated with an increase in proportion of the sheep encoding ARR/XXX for all breeds.

Figure 7.10 Scatter plot showing the proportion of sheep encoding ARR/ARR compared to R_0 for Texel flocks under breeding strategy 2.

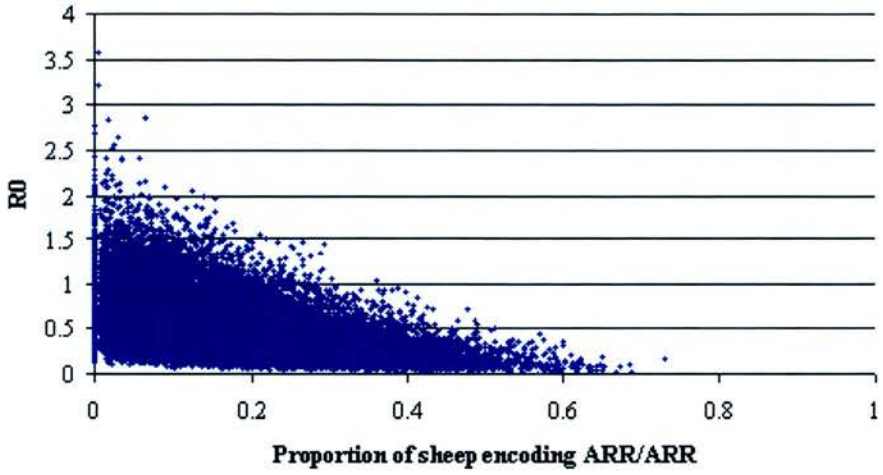
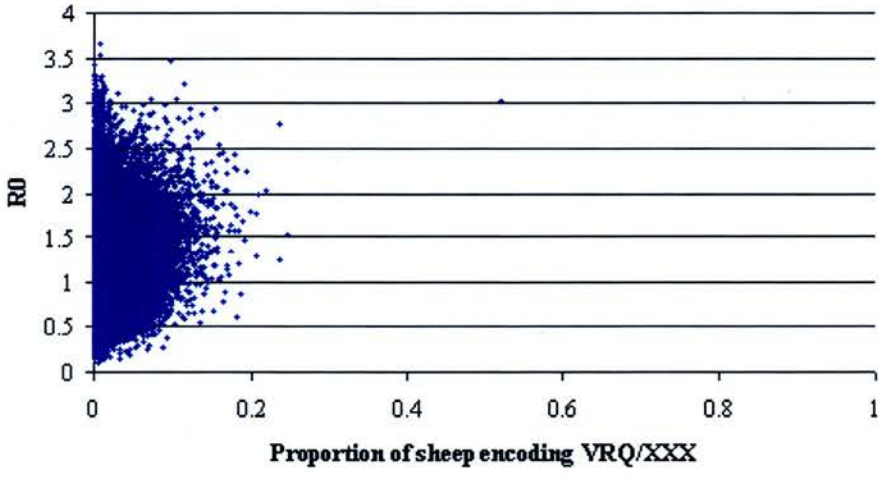


Figure 7.11 Scatter plot showing the proportion of sheep encoding VRQ/XXX compared to R_0 for Swaledale flocks under breeding strategy 1.



7.3.4 Variation in R_0

The parameter *w* was included to the expression for R_0 in order to introduce heterogeneity in the models, other than that due to variation in genotype frequencies and age structure. Incorporating this parameter widened the spread of the distribution of the R_0's, although the means remained similar. In particular, it decreased the initial proportion of flocks in which R_0 was greater than one, but increased the maximum values for R_0 (figure 7.12).

Figure 7.12 A comparison of the distribution of the R_0 values in year 14 in the original and heterogeneous models (a) Charollais (b) Texel (c) Swaledale. These graphs are taken from the simulation in which no selection was applied

(a)

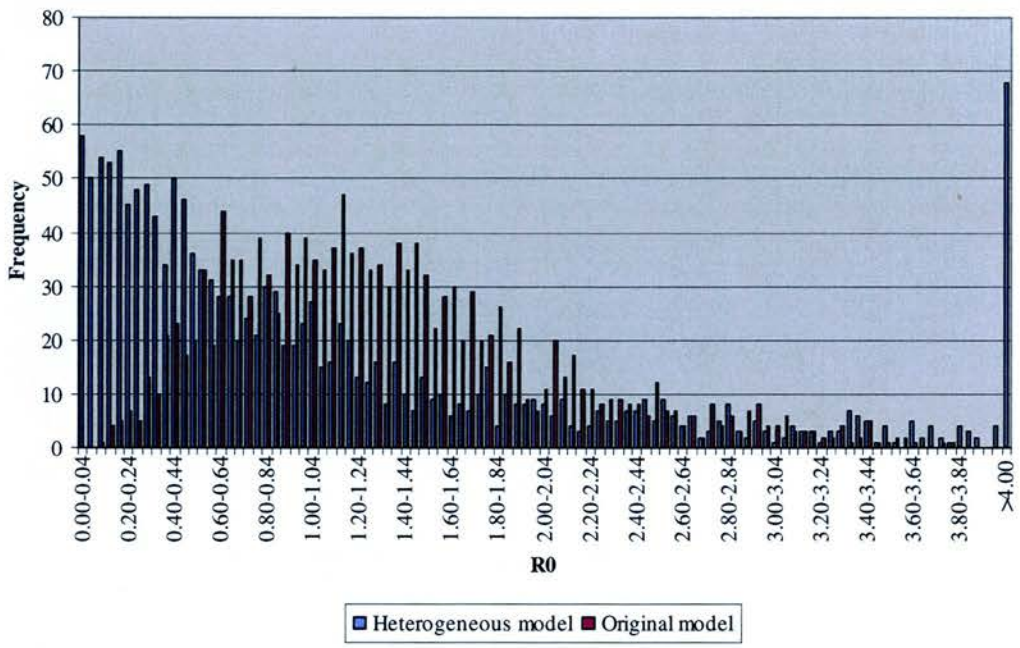
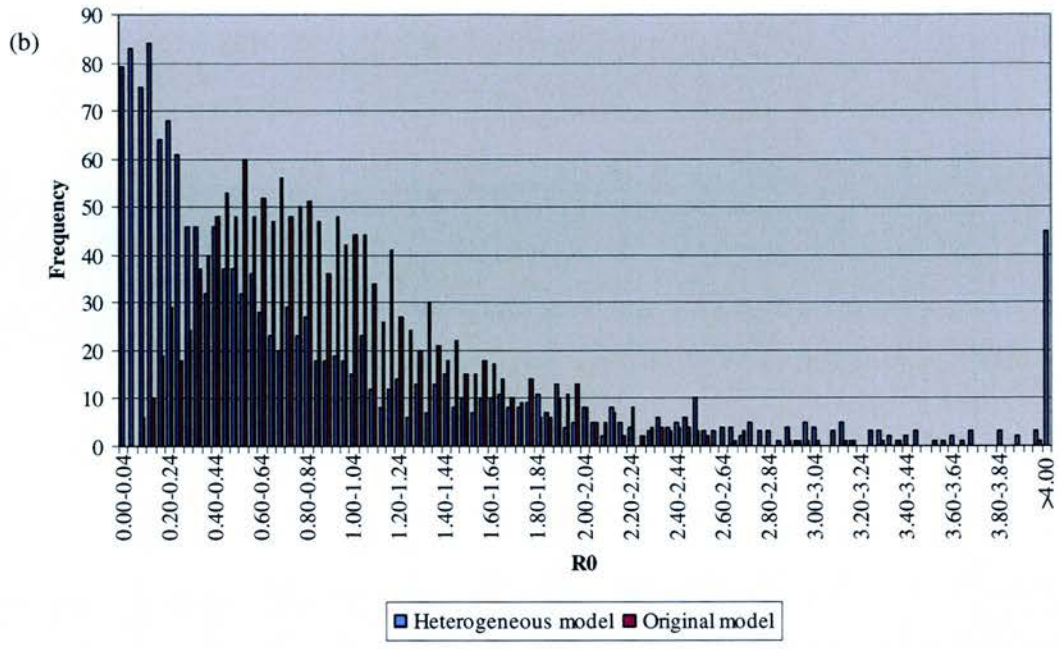
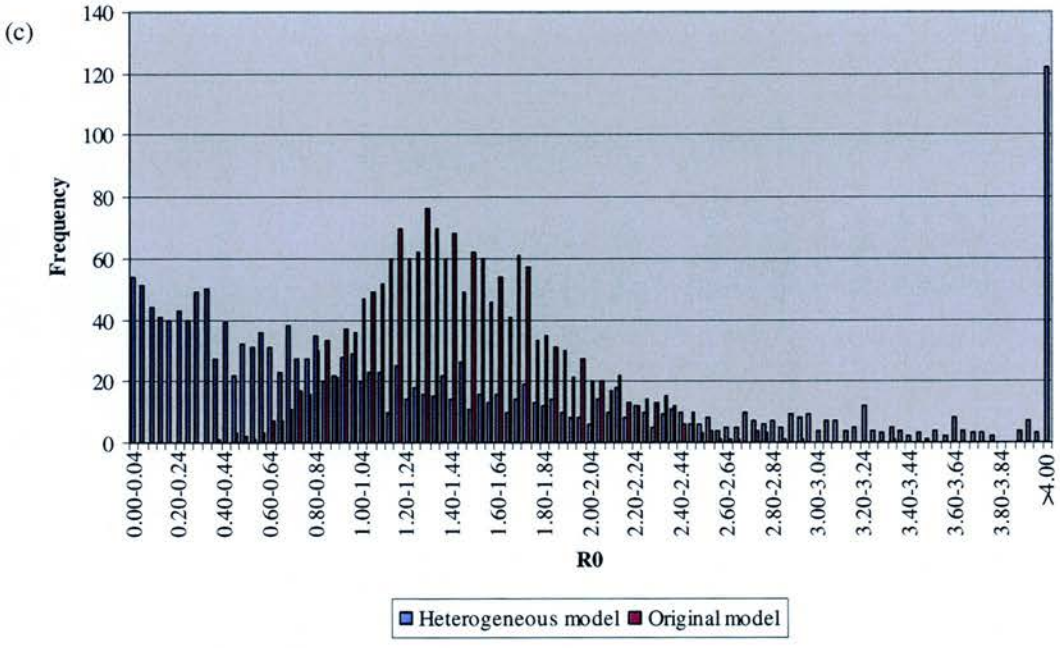


Figure 7.12 (cont)





The variation in R_0 was important in the impact of the breeding strategies. In the Charollais flocks, under breeding strategy 1, about the same period of time was required to reach the maximum reduction in $f(R_0>1)$; but under strategy 2, it took

slightly longer (two years difference) (figure 7.13). Similarly, in the Texel flocks, the NSP-based selection strategies also required a slightly longer period of time (one to two years difference) to achieve their maximum effect (figure 7.14). In the Swaledale flocks, as for the Charollais and Texel flocks, the timescales required to achieve maximum reduction in the number of flocks with an R_0 greater than one were affected. The maximum effect of breeding strategy 1 was reached about four years earlier; and reached about two years later under strategy 2 (figure 7.15). In all models, the 'extreme' breeding strategy still reduced the number of flocks with a R_0 greater than one to zero but over a slightly longer period of time (between one to three years).

Figure 7.13 A comparison of the percentages of Charollais flocks with an R_0 greater that one before and after the application of the parameter *w*.

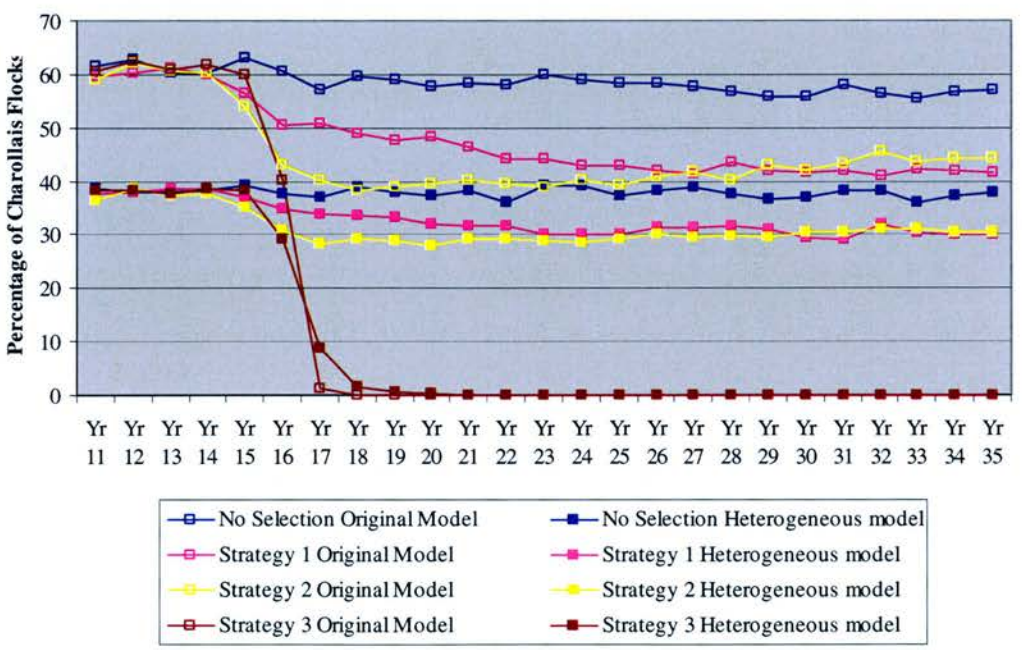


Figure 7.14 A comparison of the percentages of Texel flocks with an R_0 greater that one before and after the application of the parameter *w*.

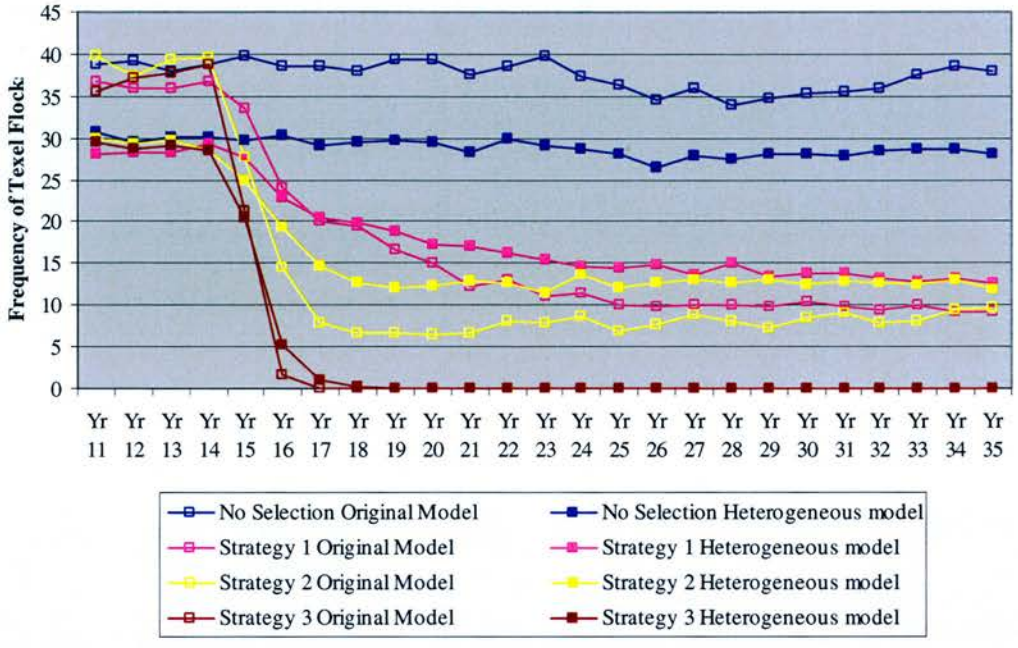
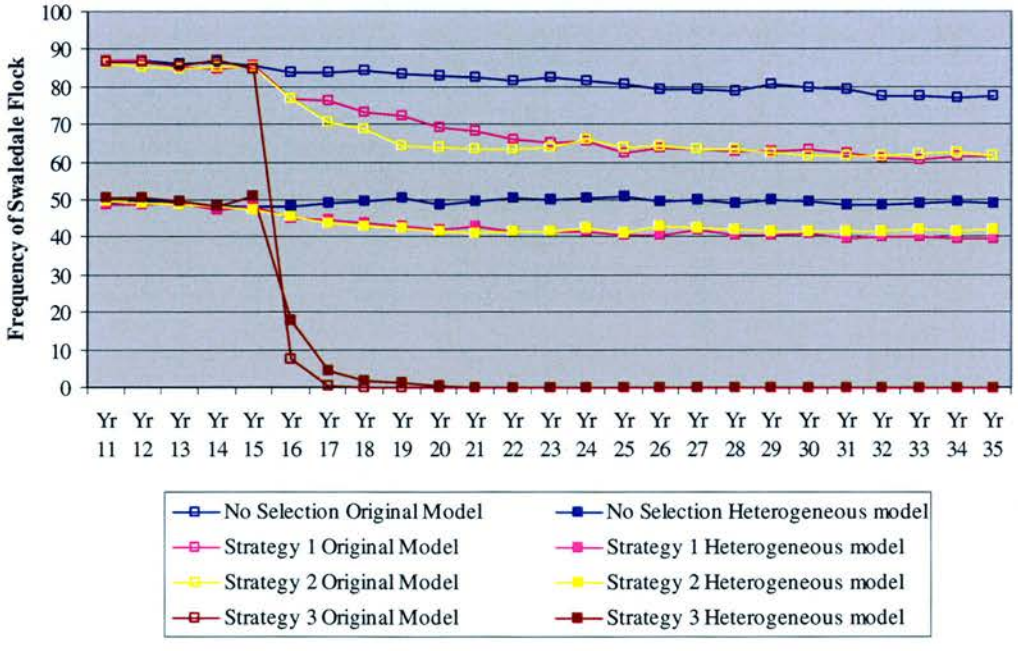


Figure 7.15 A comparison of the number of Swaledale flocks with an R_0 greater that one before and after the application of the parameter *w*.



In addition to the effect of heterogeneity on the time taken to achieve the maximum reduction in the number of flocks with an R_0 greater than one, the effectiveness of this reduction was also lowered for the two NSP-based breeding strategies in all the breeds considered, with the percentage reduction in the number of flocks with an R_0 greater than one was lower in the heterogeneous models (table 7.6). However, it should also be noted that $f(R_0>1)$ was typically lower for the heterogenous model than for the original model, principally because the initial $f(R_0>1)$ was lower (figures 7.13-7.15; table 7.6).

Table 7.6 The reduction in the number of flocks with an R_0 greater than one.

		Proportion of flocks with an R_0 greater than one at year 35			Percentage decrease	
Breed	Model	Wild-type	Breeding strategy 1	Breeding strategy 2	Breeding strategy 1	Breeding strategy 2
Charollais	Original	0.57	0.42	0.44	27%	23%
	Heterogeneous*	0.38	0.30	0.30	21%	20%
Texel	Original	0.38	0.09	0.10	76%	74%
	Heterogeneous	0.28	0.13	0.12	55%	58%
Swaledale	Original	0.78	0.61	0.62	21%	20%
	Heterogeneous	0.49	0.40	0.42	19%	15%

* i.e. the model incorporating the parameter w

7.4 Discussion

The model for R_0 does not take into account all parameters involving the establishment of scrapie within a flock, and, in particular, it does not consider the probability of a flock getting an initial infection. Rather, it focuses on whether or not an outbreak could occur if a flock did get an initial infection. When R_0 is greater than

one, a larger outbreak is possible, though even if R_0 is less than one sporadic cases may still occur.

In this chapter, it has been shown that effect of selecting only against the VRQ allele (strategies 1 and 2) depends on the frequencies of other genotypes within a breed. Crucially, in such cases the proportion of flocks with an R_0 greater than one, $f(R_0 > 1)$, is not driven to zero, even after 20 years of selection. This is most likely due to the fact that alleles associated with some susceptibility to scrapie are still maintained within the breeding population, despite the frequency of the VRQ allele being reduced to zero. Consequently, selection against the VRQ allele alone will not eliminate the risk of scrapie.

By contrast, a severe breeding strategy which actively selected for ARR/ARR (strategy 3) was able to rapidly reduce $f(R_0 > 1)$ to zero. However, this introduces other complications. In some rare breeds, the frequency of resistant sheep is so low that such a restrictive strategy could result in their extinction (Townsend *et al.*, 2005). Additionally, even if extinction was not to occur, an increase in inbreeding may occur if the genetic diversity of the rams used to sire the flock was too restricted. This is possible in some breeds of sheep (especially hill breeds) which have a lower proportion of ARR-bearing sheep (Eglin *et al.*, 2005).

However, there are two further considerations with regards to the eradication of scrapie. First, it may not be necessary to completely eliminate alleles associated with susceptibility to scrapie, and, second, the estimated genotype susceptibilities may be

much lower than those used in this chapter, which would affect the calculated flock R_0's. The results in section 7.3.3 show that the entire flock does not have to be completely resistant to scrapie in order for R_0 to fall below one (figure 7.10), so selection strategies do not need to eliminate all susceptible genotypes to eliminate the risk of a large scrapie outbreak occurring within the flock. This is an important result with regards to the concerns about the use of a restricted of gene pool and potential inbreeding which might occur when restricting the number of rams (and potentially ewes) used for breeding. However, this section also shows that even if the VRQ allele is completely eliminated from the flocks, R_0 does not necessarily fall below one (figure 7.11). This is likely due to a complementary increase in the frequencies in other alleles, including those which are associated with some susceptibility to scrapie (figures 7.7-7.9), as was also shown by Roden et al. (2006).

The estimates of susceptibilities of the genotypes given by Touzeau *et al.* (2006) are much higher than other published in the literature (Baylis *et al.*, 2004; Gubbins and Roden, 2006; Tongue *et al.*, 2006). For example, while the susceptibility estimate for ARR/ARH is still zero, that for ARR/ARQ is 0.0006 and that for ARH/ARH is 0.004 (Gubbins and Roden, 2006). These are much smaller than the susceptibility estimates used in this chapter (0.029 and 0.020, respectively; table 7.5). This suggests that the model used in this chapter may underestimate the impact of changes in the genotype profiles on R_0, and, consequently, further exclusions of genotypes from the breeding pool would not be necessary.

Apart from high estimates of genotype susceptibilities, there may also be differences in breed susceptibility. In the models presented here, the estimates are based on data from the Romanov breed, which were then extrapolated to Charollais, Texel and Swaledale breeds. It was assumed that scrapie affected the same genotypes to a similar extent, that is, the relative susceptibilities of each genotype were similar across the breeds. This was considered to be a fairly safe assumption as the breeds considered in this chapter appeared to have the similar patterns of scrapie as Romanov sheep (Baylis and Goldmann, 2004).

The variation introduced to the model in section 7.3.4 increases the range of R_0 values dramatically, although despite this increase, the introduced variation reduces the probability that a given flock will have an R_0 greater than one (where no selection for *PrP* genotype is occurring). However, the heterogeneity in the models also reduces the effectiveness of the three breeding strategies, generally by increasing the time taken to achieve the maximum reduction in the number of farms with an R_0 greater than one, and in the case of the NSP-based strategies, by lowering the percentage decrease of $f(R_0 > 1)$ achieved (table 7.6). This reduction in effectiveness is the result of an increased number of flocks with higher R_0 values than were present in the original models.

In conclusion, based on the susceptibilities used in this chapter, the models have shown that selection against the VRQ allele alone will not necessarily result in eliminating the risk of a scrapie outbreak from a flock. An 'extreme' breeding strategy has been suggested which would achieve this within a few years, but this

would involve the use of a very restricted range of genotypes, which could result in problems such as inbreeding, which has already been shown to occur even with only minimal restrictions in the breeding strategies. However, before these models can be interpreted on a national basis further work is required. In particular, the model is flock based and does not consider the distribution or the impact of the spread of scrapie nationally. The model also used high estimates of genotype susceptibilities, and did not take into account the impact of different strains of scrapie which target other alleles.

8 General Discussion

The studies in this thesis have covered several approaches to investigating the hypothesis that scrapie-susceptible sheep are more productive, a hypothesis driven by past anecdotal reports (Parry, 1962; Steele, 1964), and claimed to be true by many sheep farmers. If this hypothesis were true, it may explain the persistence of susceptible alleles over the years, and any selection for more resistant genotypes (National Scrapie Plan breeding schemes) may have an economic impact as well as an impact on the relative fitness of the sheep

It may be that selection is acting against alleles associated with susceptibility to scrapie, but because the selection pressure is very weak, it is not immediately obvious (Hoinville *et al.*, 2000; Woolhouse *et al.*, 2001; Gubbins, 2005). Other factors may contribute to this weak selection, such as the heterogeneous mixing of the national sheep flock which allows the existence of pockets of scrapie-affected flocks to act as reservoirs of infection; and the dominance, or partial dominance, of the ARR and AHQ alleles for resistance to scrapie, so that the genotypes produced in combination with the VRQ allele (i.e. ARR/VRQ and AHQ/VRQ) are not very susceptible to disease (Detwiler and Baylis, 2003), but still contain the susceptible allele. In addition, the AHQ/VRQ genotype is more resistant to scrapie than either AHQ/AHQ or VRQ/VRQ (Detwiler and Baylis, 2003), and is an example of heterozygous advantage in resistance to scrapie.

Alternatively, there may be a selective advantage associated with *PrP* genotype, but this advantage is not linked to the productivity traits investigated in this thesis. There might also be selection against certain alleles, which is dependent on the frequency of those alleles. This is frequency dependent selection: when an allele becomes common, it is selected against, and when the frequency of an allele decreases, selection against that allele also decreases. This could explain the heterogeneity of *PrP* genotypes seen in the UK sheep population, which is present despite years of apparent selection against susceptible genotypes. Work by Slate (2005) supports this theory, that the distribution of sheep genotypes seen was determined by balancing selection.

Selection against other alleles not commonly associated with susceptibility to scrapie is likely due to the presence of different strains of scrapie arising within the sheep population. Several new strains of scrapie have been described recently, which do not appear to affect the genotypes usually associated with disease (Buschmann *et al.*, 2004; Orge *et al.*, 2004; Moum *et al.*, 2005) and it is possible that heterogeneity of genotypes seen in the UK sheep population is the result of selection against different alleles by different strains over the centuries (Bruce *et al.*, 2002). Other regions of the *PrP* coding region might also influence the patterns of susceptibility of the *PrP* genotypes. Traditionally, it has been considered that susceptibility to scrapie is determined by polymorphisms at the codons 136, 154 and 171; but recently is has been found that susceptibility to the newly identified scrapie strain, Nor98, is also influenced by a polymorphism at codon 141 (Moum *et al.*, 2005), which supports this theory.

However, it may be true that the VRQ allele does indeed confers some advantage to the sheep and that it is linked to a positive trait or traits which are being selected for by farmers (Woolhouse *et al.*, 2001), such as productivity, and this possibility is the focus of this thesis.

8.1 Analysis of results

The studies in this thesis have involved multiple analytical methods, with both continuous and categorical response variables, as well as survival data. Actual productivity parameters, such as weights and Estimated Breeding Values (EBVs), and farmers' opinions (ratings data and culling information) have been compared to *PrP* genotype; and it is farmers' opinions of their sheep which would have historically influenced which sheep are retained on farm for breeding. This is an advantage of this thesis, in that it has included different approaches to measuring productivity, as opposed to most published work which has focussed on actual performance values, such as liveweight, or milk production traits. The methods used in each of the chapters, and the general results are summarised in table 8.1.

Table 8.1 A summary of the analyses used in this thesis, and the results found.

Chapter	Trait(s) measured	Analyses performed	Significance level	Association with *PrP* genotype
3	Rating scores	Logistic regression	1%	No association
4	Culling decisions	Survival Analysis	5%	No association
5	Lamb weights	ANOVA Mixed modelling	5%	Some association – negative effect of ARR/ARR
6	Signet data: weights and EBVs	ANOVA	1%	Variable – depends on the farm and its scrapie status

In many of the analyses presented in this thesis, there were no associations between productivity and *PrP* genotype, as is the case for many other studies (table 8.2). For example, although a study on the weight of German Black-Headed Mutton sheep suggested that sheep not encoding the ARR allele had higher weights at eight weeks of age, this result was dismissed by the authors as it was based on a study which compared 93 sheep encoding the ARR allele to 6 not encoding this allele (de Vries *et al.*, 2004b). Another study on the lean growth rate of Suffolk sheep did not find any relationships between *PrP* genotype and growth rate (Prokopová *et al.*, 2002). Similarly, no associations between muscle mass or depth, or milk production traits, and *PrP* genotype were found in East Friesian milk sheep or German Black- and White-Headed Mutton sheep (de Vries *et al.*, 2004b; de Vries *et al.*, 2005). Likewise, it was determined that selection for resistant genotypes would have no effect on milk production traits in French dairy sheep breeds (Barillet *et al.*, 2002).

However, associations between productivity and *PrP* genotype were identified in some analyses presented in this thesis (tables 8.1 and 8.2). A few published studies have also detected such relationships. For example, Alexander *et al.* (2005)

investigated the effect of the polymorphisms at codon 171 on productivity in Suffolk sheep, and found that sheep not encoding arginine (R) at codon 171 on either allele had more lambs, with a higher overall weight at weaning, than those that encoded R on one allele; and that both of these groups of sheep had more lambs in a litter than RR_{171} sheep. This study suggests that in Suffolk sheep, there might be some association between *PrP* genotypes and lamb production. Brandsma and co-workers also detected some association between litter size and *PrP* genotype, and found that the ARR/ARR genotype and the VRQ allele were associated with larger litter sizes in Texel sheep (Brandsma *et al.*, 2004). Another finding was that, in Texels, the ARR/ARR genotype was associated with a lower 135-day weight, whereas the VRQ allele was associated with a higher 135-day weight. However, despite these associations, it was concluded that selection for ARR/ARR rams would not adversely affect these traits (Brandsma *et al.*, 2004; 2005).

Table 8.2 Result of some published studies, and studies in this thesis, investigating the association between *PrP* genotype and productivity to date.

Productivity Trait	*Breed investigated*	*Association with PrP genotype*	*Reference*
Milk production	French Dairy Sheep	No association	Barillet *et al.* (2002)
Lean growth rate	Suffolk	No association	Prokopová *et al.* (2002)
Litter size Lamb weights Growth rate Carcase conformation	Texel	Small positive effect of VRQ and ARR/ARR on litter size; small positive effect of VRQ and small negative effect of ARR/ARR on 135 days weight. Otherwise negligible associations.	Brandsma *et al.* (2004,2005)
Milk, wool and meat production	German black-headed mutton sheep German white-headed mutton sheep East Friesian milk sheep	No association	de Vries *et al.* (2004,2005)
Reproductive ability	Suffolk	Negative effect of R at codon 171	Alexander *et al.* (2005)
Hardiness Wool Quality Body size Conformation	Swaledale Cheviot Shetland Texel Suffolk Crossbreeds	No association	Chapter 3
Cull Records	Various	No association	Chapter 4
Weights/ growth rates	Swaledale	ARR/ARR associated with lower weights at 8 weeks of age at 5% level	Chapter 5
Signet traits	Poll Dorset Charollais Texel Suffolk Welsh Mountain	Variable results; ARR associated with both increased and reduced productivity at 1% level	Chapter 6

It is possible that an association between scrapie susceptibility and productivity is not due to the direct effect of the *PrP* gene, but instead be a result of the *PrP* gene being linked to other genes (QTL – quantitative trait loci) on the same chromosome (13 q15: Castiglioni *et al.*, 1998; Iannuzzi *et al.*, 1998) which influence productivity. However, no QTL studies have mapped 'productivity genes/loci' to the same chromosome as the *PrP* gene, apart from the 'Agouti' locus, which codes for wool pigmentation (Purvis and Franklin, 2005). Consequently, it is perhaps not surprising

that most studies, both in the published literature and in this thesis, do not find an association between *PrP* genotype and productivity. Quantitative Trait Loci for major productivity traits have been found on several other chromosomes, such as muscle depth (Suffolk sheep, chromosome 1; Texel sheep, chromosome 18) and 8-week and scan weight in Suffolk sheep (chromosome 18) (Walling *et al.*, 2004, Walling *et al.*, 2002), but there does not appear to be any association between *PrP* genotype and these QTL.

Where associations between *PrP* genotype and productivity were found to be significant in this thesis, the direction of the effects was not consistent between farms, and this was not necessarily related to whether or not a farm was classified as being scrapie-affected. For example, on scrapie-affected farms, increasing susceptibility to scrapie was associated with decreasing Signet values, both phenotypic values and EBVs (Chapter 6). Similarly, it has been found that in a scrapie-affected experimental flock, sheep susceptible to scrapie, but not clinically affected, have a reduced reproductive performance (Chase-Topping *et al.*, 2005). These findings both suggest that there might be some negative impact of subclinical scrapie reducing the performance of the sheep. However, on scrapie-free farms, where this possibility is unlikely, increased susceptibility to scrapie was associated with both increased productivity and decreased productivity (for example the scrapie-free farms in Chapter 6 and the farm involved in the Longitudinal Lamb study, Chapter 5). This suggests that any associations previously seen between *PrP* genotype and productivity were farm-specific, and potentially specific to those particular families of sheep, which may explain why evidence for scrapie-susceptible

sheep being more productive is so limited, and hard to detect. These farm-specific associations may have arisen if there was some association between *PrP* genotype and productivity present by chance in the sheep chosen to establish these flocks, which was then retained within the flock by a degree of inbreeding (founder effect). What is obvious is that, despite claims by farmers, it is not possible to determine the susceptibility of sheep based on performance alone, and it is unclear why this is still believed to be possible by so many farmers.

Chapter 7 presented a model for the relationship between the basic reproductive number, R_0, and flock PrP genotype profile. The response of R_0 to various breeding strategies was used to provide an indication of the amount of selection on *PrP* genotype that would be required to drive R_0 below one. Under the current National Scrapie Plan breeding guidelines (DEFRA, 2005b), that is, an emphasis on selection against the VRQ allele only, the risk of scrapie is not eliminated, and even after 20 years of selection there is still a number of flocks which have an R_0 value greater than one. This suggests that in order to further reduce the risk of scrapie, other genotypes need to be excluded from the breeding population, although this raises complications such as inbreeding. However, it is also apparent that a flock does not have to be completely resistant to scrapie in order to reduce to risk of scrapie. In the three breeds considered here (Charollais, Texel and Swaledale), it was possible for a flock to have an R_0 less than one with as little as 20% of a flock encoding ARR/ARR. This is important, as it may reduce the potential for inbreeding, which would arise from the use of too restricted a breeding pool.

In addition, the R_0 values calculated in this thesis may be higher than in reality, as the models employed high estimates of genotype susceptibilities. This means that the number of flocks with an R_0 greater than one following selective breeding would actually be much lower than estimated here. In this case, selection against the VRQ allele may be more successful at reducing the risk of scrapie than predicted by the model.

Finally, the model presented in Chapter 7 also shows that if there is variation in the flocks' reproductive number not associated with the age and genotype profile of a flock, the effectiveness of these breeding strategies is reduced, generally by increasing the time taken to achieve the maximum reduction in the number of farms with an R_0 greater than one, and in the case of the NSP-based strategies, by lowering the maximum decrease of the number of flocks with an R_0 greater than one achieved.

8.2 Statistical versus biological significance

In all the studies where an association between *PrP* genotype and productivity has been observed, the differences in phenotypic performance between the genotypes have been very small. For example, in Chapters 5 and 6, differences in weight were detected at eight weeks, but these differences were only around 0.5 to 1.5kg. This is compared to an overall mean weight of around 13kg for the Swaledale sheep (Chapter 5), and 20kg for the Poll Dorset sheep (Chapter 6). These differences in weight between the genotypes may be so small that they may not be easily detectable to the farmer on initial visual inspection, and even if they were, the differences between the genotypes are no longer apparent at later ages (Scan age, Chapter 5 and

6, Mature age Chapter 6) when the lambs are sold, so the impact of the NSP on the productivity of the UK sheep flocks should be minimal, and not be of concern.

However, the analysis of the Signet EBVs suggests the opposite, with the differences between some of the genotype groups being large. For example, the difference in mean maternal ability EBV between R1 and R5 sheep on farm H19 is 0.302: on average R1 sheep have 40% of the EBV of R5 sheep. The magnitude of this difference is more likely to be of interest to the farmer, and more important economically, as any selection for ARR could influence the value of the flock.

8.3 Other consequences

As previously mentioned, the aim of the NSP is to increase the frequency of sheep resistant to scrapie, and potentially BSE, in the national sheep flock, and thus reduce the possible human health risk posed if indeed BSE is present in sheep. The NSP provides guidelines to achieve this aim regarding the use of rams and ewes of each genotype when breeding. On farms with a confirmed case of scrapie, either the entire flock is genotyped and all rams not encoding ARR/ARR, and all ewes not encoding ARR/xxx (except ARR/VRQ) are culled with compensation depending on genotype (no compensation for ARR/xxx rams and ARR/VRQ ewes); or the entire flock is culled (DEFRA,2005b; 2005b).

Based on the results in this thesis, farmers' concerns that the NSP would have a negative economic impact on the sheep industry appear to be unfounded, because the effects seen are either very small or limited to a very few farms. However, there may

be other drawbacks to the selection for resistant genotypes. As previously mentioned, an increased incidence of inbreeding may occur if the genetic diversity of the rams used to sire the flock is too restricted, as might occur in some of the hill breeds, many of which have a relatively low frequency of NSP types 1 & 2 and higher frequency of NSP type 3 sheep (Eglin *et al.*, 2005). This in itself may reduce productivity by producing inbred sheep which are not very hardy, do not perform well and are more susceptible to problems such as parasites and lameness. Furthermore, in some rare breeds, the frequency of resistant sheep is so low that restricting the breeding of susceptible sheep could result in their extinction, which has been accounted for in breeding strategies for these breeds (Townsend *et al.*, 2005). Finally, another concern about breeding a ARR- homogenous sheep population is that, theoretically, a major epidemic could occur if a strain of scrapie arises which targets the ARR allele.

8.4 Further work

This thesis has not assessed the impact of *PrP* genotype on all possible productivity parameters, nor has it included any studies on health traits. Associations between *PrP* genotype and what are essentially measures of the health of a sheep have rarely been studied. Further work could include investigating the effects of scrapie susceptibility on other chronic or recurrent diseases and problems, and any disease chosen for further study should have well-recognised clinical signs and clinical progression; so that any changes in health status can be easily monitored. Possibilities for study are footrot; or whether increased susceptibility to scrapie is associated with increased resistance to parasitism, especially as QTL on chromosomes 2, 3, 14 and 20 have been identified in Swaledale sheep which are

associated with resistance to gastrointestinal parasites (Davies *et al.*, 2006). The study involving Signet data (Chapter 6) did touch on parasitism, by way of analysing FEC and FEC 2 EBVs (Faecal Egg Count EBV for two different types of worms). However, no significant associations were seen at the 1% level between these parameters and *PrP* genotype. More direct measures of parasitism could also be studied, such as actual egg counts, and post-mortem number of species present and locations of these species (in the case of aberrant migration of larvae).

There is also a lack of cohesion between the farms used in the different studies, so the results of the farms could not be compared across these studies, as more likely than not, a farm was only involved in one area of the study. This could have been especially useful in comparing the results from Chapters 3 and 4, where subjective opinions were assessed in both cases, to see if sheep rated by the farmer as below average in certain traits were selected for culling. The lack of cohesion may be corrected by using a number of large farms involving cross- and pure-bred sheep; both matched (in terms of breed, flock size, and region) scrapie-affected and scrapie-free, and matched scrapie-free farms, in all areas of the study over several years, and should be considered for any further work.

In all cases, further studies need to involve scrapie-free farms as this study has shown that scrapie does negatively affect productivity in susceptible sheep. Additionally, further work assessing the relationship between susceptible *PrP* genotypes and sheep productivity on farms may not be possible in the UK, due to the loss of scrapie susceptible alleles through the implementation of the NSP. Consequently, studies

may need to be performed in countries without scrapie, but with the full range of common genotypes, such as Australia or New Zealand.

8.5 Conclusion

In summary, it is still unclear what has been influencing the persistence of scrapie susceptible genotypes within the UK sheep population. The lack of consistent association between productivity and *PrP* genotype, both in published work and within this thesis, suggests that overall, scrapie-susceptible sheep are not more productive, or that any difference in productivity attributable to *PrP* genotype is sufficiently small that the studies to date are not able to detect them. If the latter is the case, then it is unlikely that these differences will be of any importance.

However, elimination of scrapie from the UK is unlikely to occur in the near future, as the models in this thesis show that with the current breeding strategies, the risk of a scrapie outbreak will never reach zero, and other models suggest that with controlled breeding for resistant genotypes, the elimination of scrapie will take decades (Gubbins and Webb, 2005; Gubbins and Roden, 2006), with the appearance of any new strains prolonging the time taken for this eradication.

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Appendices

Appendix 1 Analysis of deviance tables with p-values for the effects of PrP genotype on the traits measured

In order to derive a *p-value* for each of the *PrP* genotype groups, once the minimal model was obtained, these terms were replaced into the model as the last term, and an Analysis of Deviance table obtained in each case. These tables are presented in this appendix, and are listed by trait and then by Farm. In some cases there are variations in the final number of sheep analyzed. This is due to some sheep being of unknown age, and thus being excluded from a model including Age group. i) Risk group; ii) Allelic group iii) ERA group. Pr(Chi) – significance of the term from χ^2 tables.

1.1 Hardiness

D55

a i)

Term	Df	Deviance	Pr(Chi)
Status	1	7.85	0.005
Age group	1	10.00	0.002
Risk group	4	1.99	0.738
Residuals	180	236.06	
Total	186	255.88	

ii)

Term	Df	Deviance	Pr(Chi)
Status	1	7.85	0.005
Age group	1	10.00	0.002
Allelic group	3	1.30	0.728
Residuals	180	236.74	
Total	186	255.88	

iii)

Term	Df	Deviance	Pr(Chi)
Status	1	7.85	0.005
Age group	1	10.00	0.002
ERA group	3	0.70	0.873
Residuals	180	237.34	
Total	186	255.88	

D66

b i)

Term	Df	Deviance	Pr(Chi)
Risk group	3	3.17	0.367
Residuals	65	78.05	
Total	68	81.22	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	1	0.00	0.983
Residuals	67	81.22	
Total	68	81.22	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	2	0.42	0.812
Residuals	66	80.80	
Total	68	81.22	

J58

c i)

Term	Df	Deviance	Pr(Chi)
Breed	3	9.23	0.026
Risk group	4	0.57	0.967
Residuals	295	409.85	
Total	302	419.65	

ii)

Term	Df	Deviance	Pr(Chi)
Breed	3	9.23	0.026
Allelic group	3	1.79	0.617
Residuals	296	408.63	
Total	302	419.65	

iii)

Term	Df	Deviance	Pr(Chi)
Breed	3	9.23	0.026
ERA group	3	1.15	0.765
Residuals	296	409.27	
Total	302	419.65	

M38

d i)

Term	Df	Deviance	Pr(Chi)
Age group	1	5.21	0.022
Risk group	4	0.50	0.973
Residuals	65	78.71	
Total	70	84.43	

ii)

Term	Df	Deviance	Pr(Chi)
Age group	1	5.21	0.022
Allelic group	3	0.82	0.844
Residuals	66	76.21	
Total	70	84.43	

iii)

Term	Df	Deviance	Pr(Chi)
Age group	1	5.21	0.022
ERA group	3	0.54	0.910
Residuals	66	76.21	
Total	70	84.43	

N5457

e i)

Term	Df	Deviance	Pr(Chi)
Age group	1	8.56	0.003
Risk group	4	8.30	0.081
Residuals	161	214.17	
Total	166	231.03	

ii)

Term	Df	Deviance	Pr(Chi)
Age group	1	8.56	0.003
Allelic group	3	2.55	0.467
Residuals	162	219.92	
Total	166	231.03	

iii)

Term	Df	Deviance	Pr(Chi)
Age group	1	8.56	0.003
ERA group	3	4.44	0.218
Residuals	162	218.03	
Total	166	231.03	

1.2 Wool Quality

D55

a i)

Term	Df	Deviance	Pr(Chi)
Status	1	9.14	0.002
Risk group	4	1.74	0.784
Residuals	181	246.81	
Total	186	257.69	

ii)

Term	Df	Deviance	Pr(Chi)
Status	1	9.14	0.002
Allelic group	3	1.33	0.721
Residuals	182	247.21	
Total	186	257.69	

iii)

Term	Df	Deviance	Pr(Chi)
Status	1	9.14	0.002
ERA group	3	1.27	0.735
Residuals	182	247.27	
Total	186	257.69	

D66

b i)

Term	Df	Deviance	Pr(Chi)
Risk group	3	1.26	0.738
Residuals	65	75.79	
Total	68	77.05	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	1	0.90	0.342
Residuals	67	76.14	
Total	68	77.05	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	2	1.24	0.538
Residuals	66	75.81	
Total	68	77.05	

J58

c i)

Term	Df	Deviance	Pr(Chi)
Breed	3	11.50	0.009
Age group	1	7.58	0.006
Risk group	4	2.09	0.719
Residuals	282	349.28	
Total	290	370.45	

ii)

Term	Df	Deviance	Pr(Chi)
Breed	3	11.50	0.009
Age group	1	7.58	0.006
Allelic group	3	6.04	0.109
Residuals	283	345.33	
Total	290	370.45	

iii)

Term	Df	Deviance	Pr(Chi)
Breed	3	11.50	0.009
Age group	1	7.58	0.006
ERA group	3	2.30	0.513
Residuals	283	349.07	
Total	290	370.45	

M38

d i)

Term	Df	Deviance	Pr(Chi)
Breed	1	10.61	0.001
Risk group	4	3.41	0.492
Residuals	97	118.01	
Total	102	132.03	

ii)

Term	Df	Deviance	Pr(Chi)
Breed	1	10.61	0.001
Allelic group	3	2.37	0.500
Residuals	98	119.06	
Total	102	132.03	

iii)

Term	Df	Deviance	Pr(Chi)
Breed	1	10.61	0.001
ERA group	3	3.21	0.361
Residuals	98	118.21	
Total	102	132.03	

N5457

e i)

Term	Df	Deviance	Pr(Chi)
Age group	1	11.96	0.001
Risk group	4	2.64	0.619
Residuals	161	185.74	
Total	166	200.34	

ii)

Term	Df	Deviance	Pr(Chi)
Age group	1	11.96	0.001
Allelic group	3	1.58	0.663
Residuals	162	186.80	
Total	166	200.34	

iii)

Term	Df	Deviance	Pr(Chi)
Age group	1	11.96	0.001
ERA group	3	2.95	0.400
Residuals	162	185.44	
Total	166	200.34	

S56

f i)

Term	Df	Deviance	Pr(Chi)
Risk group	4	3.75	0.441
Residuals	25	32.90	
Total	29	36.65	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	3	0.36	0.949
Residuals	26	36.29	
Total	29	36.65	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	3	1.76	0.624
Residuals	26	34.90	
Total	29	36.65	

S62

g i)

Term	Df	Deviance	Pr(Chi)
Risk group	4	7.08	0.132
Residuals	86	118.53	
Total	90	125.61	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	3	7.48	0.058
Residuals	87	118.13	
Total	90	125.61	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	3	3.37	0.338
Residuals	87	122.25	
Total	90	125.61	

1.3 Conformation

D55

a i)

Term	Df	Deviance	Pr(Chi)
Breed	3	10.01	0.018
Age group	1	7.20	0.007
Risk group	4	2.69	0.611
Residuals	178	237.79	
Total	186	257.69	

ii)

Term	Df	Deviance	Pr(Chi)
Breed	3	10.01	0.018
Age group	1	7.20	0.007
Allelic group	3	1.59	0.661
Residuals	179	238.88	
Total	186	257.69	

iii)

Term	Df	Deviance	Pr(Chi)
Breed	3	10.01	0.018
Age group	1	7.20	0.007
ERA group	3	3.70	0.296
Residuals	179	236.78	
Total	186	257.69	

D66

b i)

Term	Df	Deviance	Pr(Chi)
Risk group	3	1.41	0.703
Residuals	65	59.13	
Total	68	60.54	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	1	0.05	0.827
Residuals	67	60.49	
Total	68	60.54	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	2	0.01	0.996
Residuals	66	60.53	
Total	68	60.54	

J58

c i)

Term	Df	Deviance	Pr(Chi)
Breed	3	11.53	0.009
Risk group	4	5.28	0.259
Residuals	295	403.07	
Total	302	419.89	

ii)

Term	Df	Deviance	Pr(Chi)
Breed	3	11.53	0.009
Allelic group	3	1.14	0.767
Residuals	296	407.21	
Total	302	419.89	

iii)

Term	Df	Deviance	Pr(Chi)
Breed	3	11.53	0.009
ERA group	3	1.14	0.767
Residuals	296	407.21	
Total	302	419.89	

M38

d i)

Term	Df	Deviance	Pr(Chi)
Risk group	4	2.42	0.660
Residuals	98	133.21	
Total	102	135.63	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	3	0.57	0.903
Residuals	99	135.06	
Total	102	135.63	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	3	1.31	0.727
Residuals	99	134.32	
Total	102	135.63	

N5457

e i)

Term	Df	Deviance	Pr(Chi)
Age group	1	6.29	0.012
Risk group	4	4.89	0.299
Residuals	161	199.03	
Total	166	210.21	

ii)

Term	Df	Deviance	Pr(Chi)
Age group	1	6.29	0.012
Allelic group	3	6.83	0.078
Residuals	162	197.09	
Total	166	210.21	

iii)

Term	Df	Deviance	Pr(Chi)
Age group	1	6.29	0.012
ERA group	3	6.85	0.077
Residuals	162	197.07	
Total	166	210.21	

S56

f i)

Term	Df	Deviance	Pr(Chi)
Risk group	4	7.90	0.095
Residuals	25	30.29	
Total	29	38.19	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	3	4.08	0.253
Residuals	26	34.11	
Total	29	38.19	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	3	2.56	0.465
Residuals	26	35.63	
Total	29	38.19	

S62

g i)

Term	Df	Deviance	Pr(Chi)
Age group	1	29.09	<0.001
Risk group	4	0.56	0.968
Residuals	84	94.72	
Total	89	124.37	

ii)

Term	Df	Deviance	Pr(Chi)
Age group	1	29.09	<0.001
Allelic group	3	0.30	0.960
Residuals	85	94.98	
Total	89	124.37	

iii)

Term	Df	Deviance	Pr(Chi)
Age group	1	29.09	<0.001
ERA group	3	0.25	0.970
Residuals	85	95.03	
Total	89	124.37	

1.4 Body Size

a i) D55

Term	Df	Deviance	Pr(Chi)
Breed	3	14.82	0.002
Age group	1	6.27	0.012
Risk group	4	0.47	0.977
Residuals	181	252.50	
Total	186	259.23	

ii)

Term	Df	Deviance	Pr(Chi)
Breed	3	14.82	0.002
Age group	1	6.27	0.012
Allelic group	3	2.42	0.490
Residuals	182	250.54	
Total	186	259.23	

iii)

Term	Df	Deviance	Pr(Chi)
Breed	3	14.82	0.002
Age group	1	6.27	0.012
ERA group	3	1.29	0.732
Residuals	182	251.68	
Total	186	259.23	

b i) D66

Term	Df	Deviance	Pr(Chi)
Risk group	3	5.30	0.151
Residuals	65	69.43	
Total	68	74.73	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	1	0.12	0.730
Residuals	67	74.61	
Total	68	74.73	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	2	0.82	0.665
Residuals	66	73.92	
Total	68	74.73	

c i) J58

Term	Df	Deviance	Pr(Chi)
Age group	1	6.57	0.010
Risk group	4	5.36	0.252
Residuals	286	378.50	
Total	290	383.86	

ii)

Term	Df	Deviance	Pr(Chi)
Age group	1	6.57	0.010
Allelic group	3	2.62	0.454
Residuals	287	381.24	
Total	290	383.86	

iii)

Term	Df	Deviance	Pr(Chi)
Age group	1	6.57	0.010
ERA group	3	1.26	0.739
Residuals	287	382.61	
Total	290	383.86	

d i) M38

Term	Df	Deviance	Pr(Chi)
Risk group	4	3.01	0.555
Residuals	98	117.51	
Total	102	120.53	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	3	4.39	0.222
Residuals	99	116.14	
Total	102	120.53	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	3	0.76	0.860
Residuals	99	119.77	
Total	102	120.53	

N5457

e i)

Term	Df	Deviance	Pr(Chi)
Risk group	4	2.66	0.616
Residuals	162	160.52	
Total	166	163.18	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	3	2.25	0.522
Residuals	163	160.93	
Total	166	163.18	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	3	2.25	0.522
Residuals	163	160.93	
Total	166	163.18	

S56

f i)

Term	Df	Deviance	Pr(Chi)
Risk group	4	3.49	0.480
Residuals	25	38.10	
Total	29	41.59	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	3	3.01	0.389
Residuals	26	38.57	
Total	29	41.59	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	3	1.97	0.579
Residuals	26	39.62	
Total	29	41.59	

S62

g i)

Term	Df	Deviance	Pr(Chi)
Risk group	4	4.60	0.331
Residuals	86	88.65	
Total	90	93.25	

ii)

Term	Df	Deviance	Pr(Chi)
Allelic group	3	1.93	0.587
Residuals	87	91.32	
Total	90	93.25	

iii)

Term	Df	Deviance	Pr(Chi)
ERA group	3	2.41	0.492
Residuals	87	90.84	
Total	90	93.25	

Appendix 2 Survival parameters

2.1 Hazard Ratios associated with YoB and Genotype

The reference YoB in each case is ≤ 1993, except for Farm U29, where it is ≤ 1994. The reference level of each genotype group is as follows: Risk group – R3; Allelic group – I; ERA group – d. On some farms, genotypes had to be combined. These are as follows: Farm D12 – Risk groups 1 and 2 were combined, as no R2 sheep were removed from the number sampled; Risk group 4 and 5 were also combined, as all R5 sheep were culled. Farm M28 – No ARR/ARR sheep were removed from the flock sampled, so Risk groups 1 and 3 were combined, and this became the reference level. Significant YoB and genotype group results are highlighted in bold. LCL – Lower 95% confidence limit; UCL – Upper 95% confidence limit. Est. HR – Estimated Hazard Ratio.

A35

	Est. HR	LCL	UCL
YoB 1994	0.53	0.17	1.62
YoB 1995	**0.10**	**0.01**	**0.79**
YoB 1996	0.27	0.03	2.20
YoB 1997	**0.20**	**0.06**	**0.67**
YoB 1998	**0.17**	**0.04**	**0.62**
YoB ≥ 1999	1.05	0.42	2.62

C16

	Est. HR	LCL	UCL
YoB 1994	**0.44**	**0.27**	**0.72**
YoB 1995	**0.44**	**0.23**	**0.83**
YoB 1996	**0.38**	**0.23**	**0.65**
YoB 1997	**0.19**	**0.10**	**0.36**

D12

	Est. HR	LCL	UCL
Risk groups 1and 2	0.38	0.08	1.90
Risk groups 4 and 5	**6.89**	**2.83**	**16.80**
Allelic group II	**14.39**	**4.99**	**41.53**
Allelic group III	**3.14**	**1.09**	**9.05**
Allelic group IV	**34.64**	**12.66**	**94.78**
ERA group a	**0.11**	**0.02**	**0.47**
ERA group b	**0.19**	**0.08**	**0.47**
ERA group c	0.58	0.26	1.33

D34

	Est. HR	LCL	UCL
Allelic group II	**1.62**	**1.02**	**2.59**
Allelic group III	2.15	0.86	5.40
Allelic group IV	1.49	0.73	3.03
ERA group a	**0.51**	**0.26**	**0.98**
ERA group b	0.69	0.35	1.33
ERA group c	0.93	0.49	1.77

H19

	Est. HR	LCL	UCL
YoB 1994	**0.39**	**0.22**	**0.70**
YoB 1995	**0.25**	**0.15**	**0.42**
YoB 1996	**0.07**	**0.03**	**0.15**
YoB 1997	**0.25**	**0.16**	**0.4**

J09

	Est. HR	LCL	UCL
YoB 1994	**0.45**	**0.23**	**0.88**
YoB 1995	**0.30**	**0.14**	**0.64**
YoB 1996	**0.31**	**0.16**	**0.61**
YoB 1997	**0.19**	**0.09**	**0.39**
Risk group 1	0.41	0.16	1.08
Risk group 4	0.84	0.48	1.46
Risk group 5	**2.97**	**1.54**	**5.71**
YoB 1994	**0.49**	**0.25**	**0.97**
YoB 1995	**0.34**	**0.16**	**0.73**
YoB 1996	**0.33**	**0.17**	**0.64**
YoB 1997	**0.20**	**0.10**	**0.41**
Allelic group II	0.54	0.23	1.26
Allelic group III	1.46	0.82	2.60
Allelic group IV	**3.61**	**1.90**	**6.86**
YoB 1994	0.53	0.27	1.03
YoB 1995	**0.31**	**0.14**	**0.65**
YoB 1996	**0.42**	**0.23**	**0.78**
YoB 1997	**0.26**	**0.13**	**0.51**
ERA group a	**0.26**	**0.10**	**0.66**
ERA group b	0.61	0.36	1.02
ERA group c	**0.27**	**0.12**	**0.61**

M28

	Est. HR	LCL	UCL
Risk group 4	1.70	0.96	3.02
Risk group 5	**3.87**	**1.47**	**10.19**
Allelic group II	2.32	0.80	6.73
Allelic group III	1.62	0.89	2.94
Allelic group IV	**3.87**	**1.47**	**10.19**

M30

	Est. HR	LCL	UCL
Risk group 1	0.62	0.15	2.59
Risk group 4	**1.72**	**1.02**	**2.88**
Risk group 5	**5.86**	**3.06**	**11.23**
Allelic group II	2.12	0.82	5.50
Allelic group III	**1.77**	**1.04**	**3.02**
Allelic group IV	**6.21**	**3.25**	**11.87**
ERA group a	0.28	0.07	1.15
ERA group b	**0.44**	**0.27**	**0.72**
ERA group c	0.86	0.37	2.03

P27

	Est. HR	LCL	UCL
ERA group a	0.52	0.13	2.10
ERA group b	**0.25**	**0.08**	**0.72**
ERA group c	**0.26**	**0.09**	**0.75**

S03

	Est. HR	LCL	UCL
YoB 1994	0.46	0.21	1.01
YoB 1995	**0.22**	**0.11**	**0.46**
YoB 1996	**0.31**	**0.15**	**0.61**
YoB 1997	**0.19**	**0.10**	**0.34**

T36

	Est. HR	LCL	UCL
YoB 1994	**0.41**	**0.19**	**0.92**
YoB 1995	**0.30**	**0.13**	**0.69**
YoB 1996	**0.22**	**0.10**	**0.50**
YoB 1997	**0.15**	**0.07**	**0.34**
YoB 1998	**0.14**	**0.07**	**0.29**
YoB ≥ 1999	**0.05**	**0.02**	**0.13**

T59

	Est. HR	LCL	UCL
Risk group 1	0.70	0.14	3.48
Risk group 2	0.24	0.03	2.01
Risk group 4	0.97	0.24	3.89
Risk group 5	**4.19**	**1.18**	**14.84**

U29

	Est. HR	LCL	UCL
YoB 1995	0.60	0.03	10.24
YoB 1996	0.61	0.04	10.55
YoB 1997	1.82	0.20	16.64
YoB 1998	7.00	0.92	53.13
YoB ≥ 1999	5.76	0.77	42.88
ERA group a	0.10	0.01	1.27
ERA group b	**0.16**	**0.03**	**0.81**
ERA group c	0.22	0.04	1.12

U29 – YoB only

	Est. HR	LCL	UCL
YoB 1995	0.97	0.06	15.30
YoB 1996	1.00	0.06	15.90
YoB 1997	2.25	0.25	20.00
YoB 1998	**8.19**	**1.10**	**61.10**
YoB ≥ 1999	6.12	0.83	45.30

Appendix 3 Associations between genotype and Signet parameters

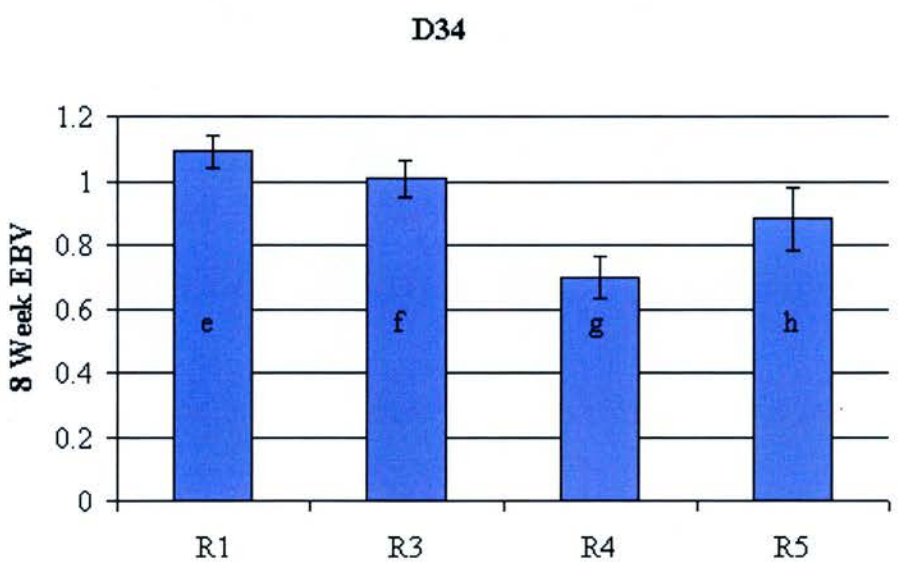
3.1 Graphs of the Significant Associations at the 1% level between the genotype groupings and the Signet parameters

The graphs are the mean value and 95% confidence interval for each genotype level. Graph bars labelled with different letters (e – h) are significantly different to each other. Individual farms are shown first, where appropriate, then the paired farms.

3.1.1 Figure 1 – Risk Group

Individual Farms

i



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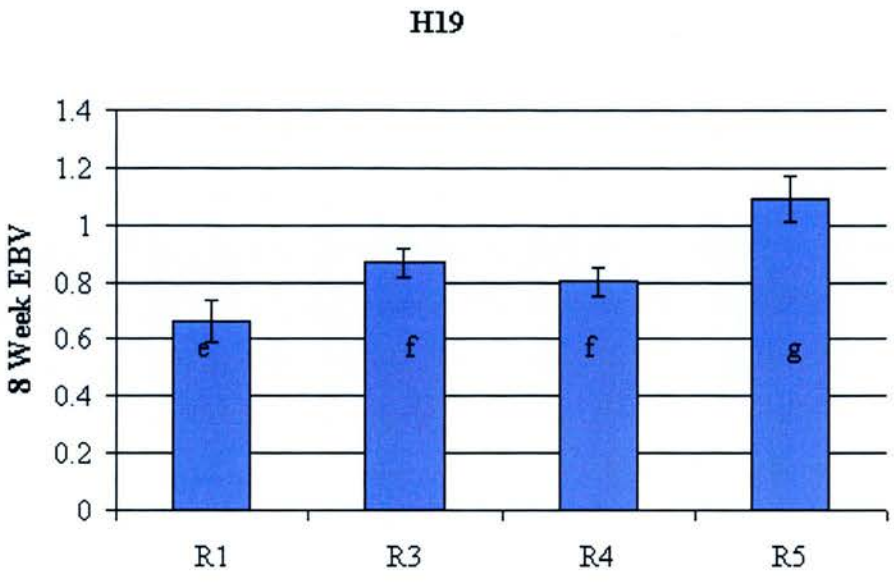
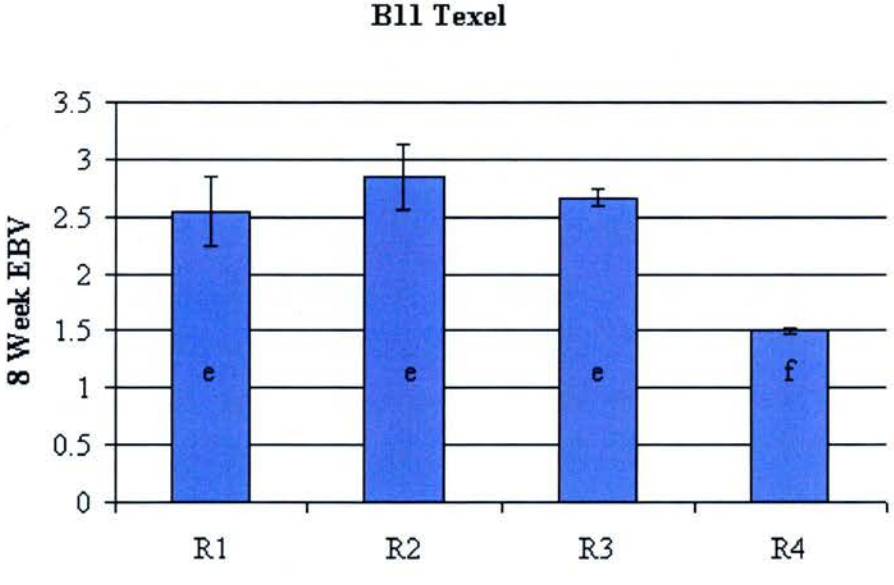
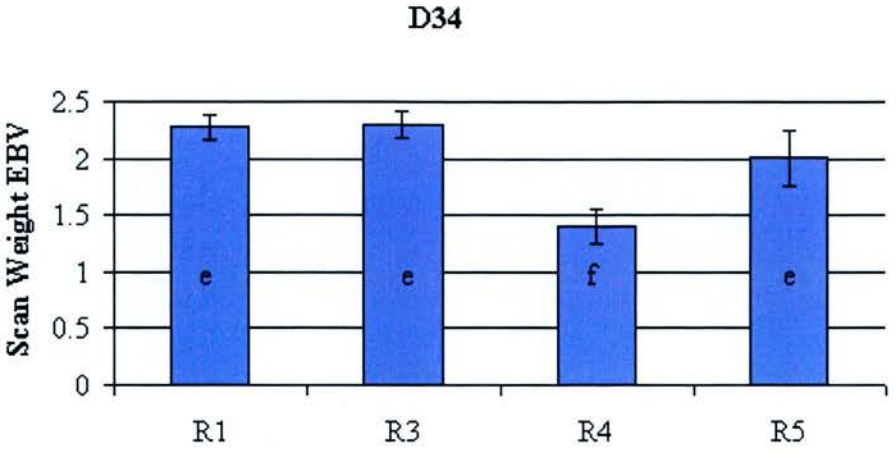

H19
8 Week EBV
1.4
1.2
1
0.8
0.6
0.4
0.2
0
e
f
f
g
R1
R3
R4
R5

iii


B11 Texel
8 Week EBV
3.5
3
2.5
2
1.5
1
0.5
0
e
e
e
f
R1
R2
R3
R4

iv


D34
Scan Weight EBV
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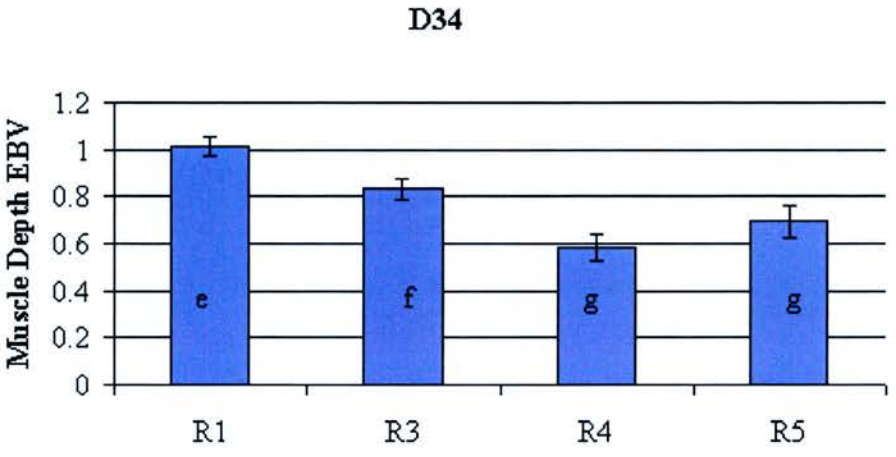
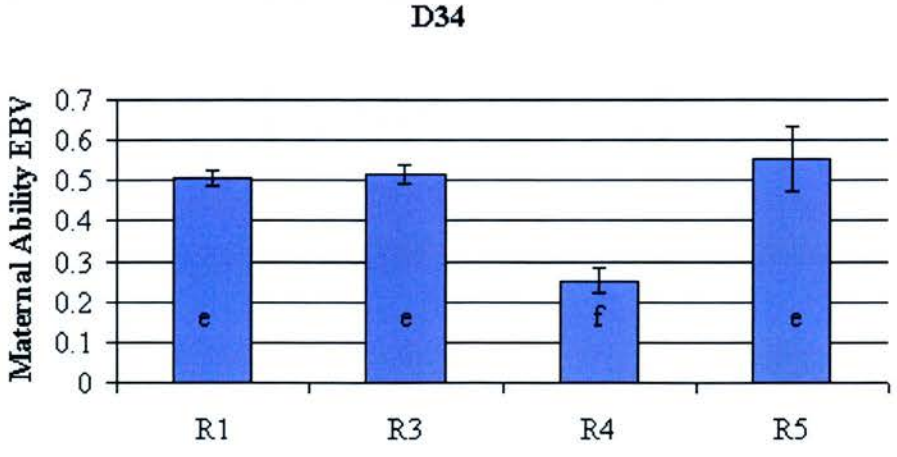
1
0.5
0
e
e
f
e
R1
R3
R4
R5

v

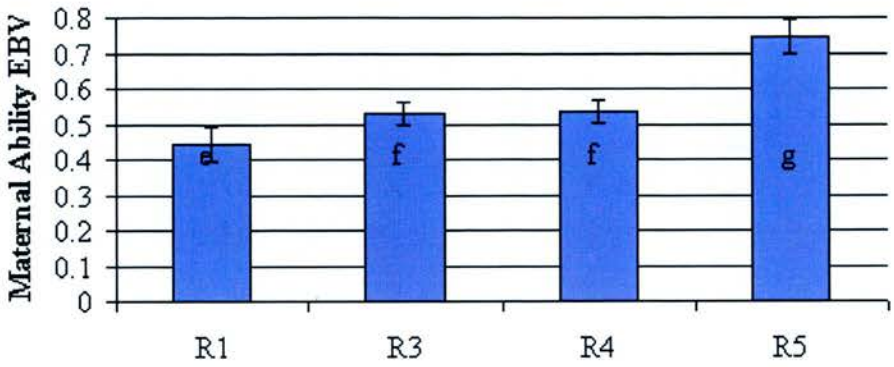

D34
Muscle Depth EBV
1.2
1
0.8
0.6
0.4
0.2
0
e
f
g
g
R1
R3
R4
R5

vi


D34
Maternal Ability EBV
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
e
e
f
e
R1
R3
R4
R5

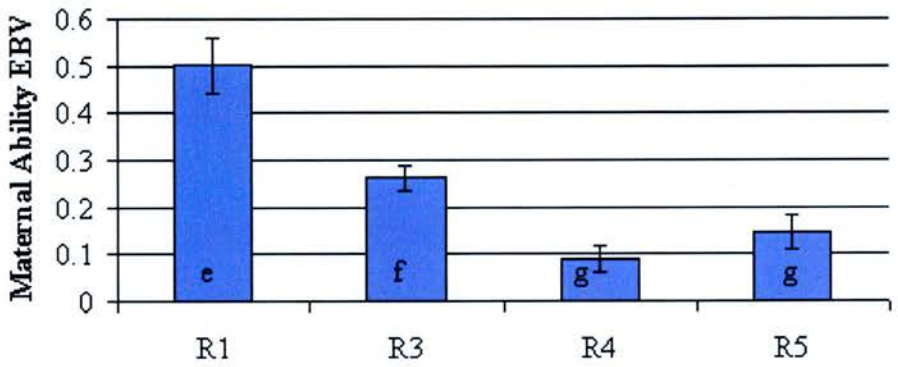
vii

H19



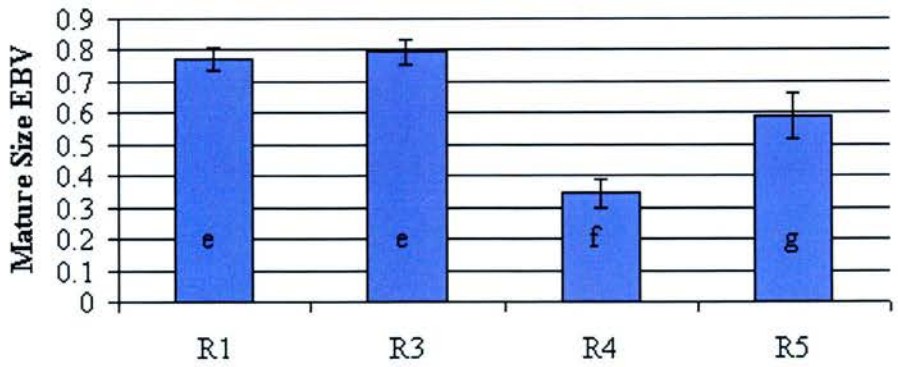
viii

S03

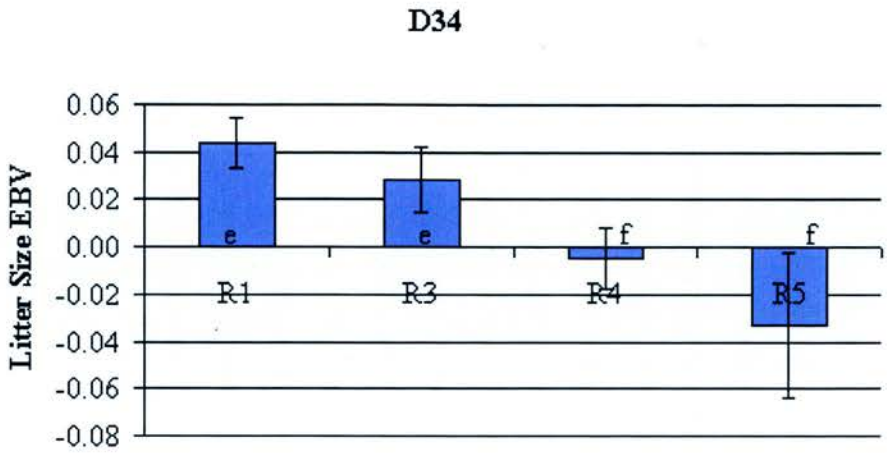


ix

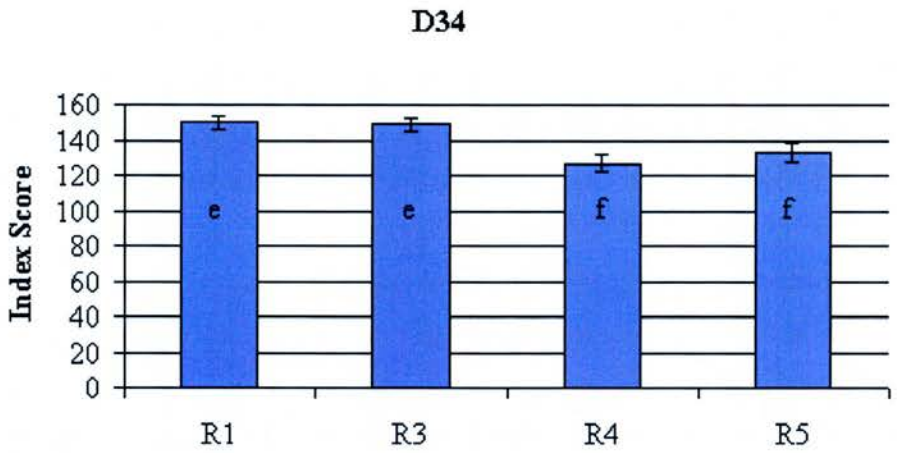
D34



x

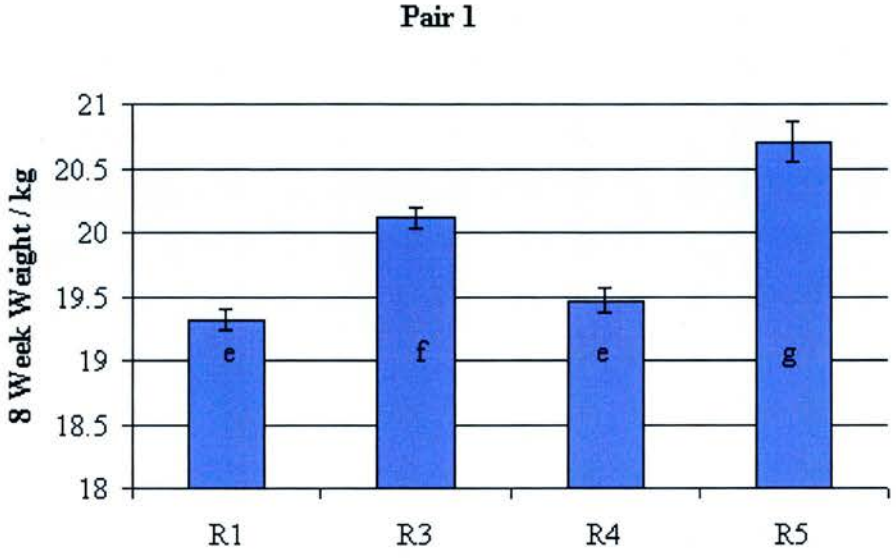

D34
Litter Size EBV
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
e
e
f
f
R1
R3
R4
R5

xi

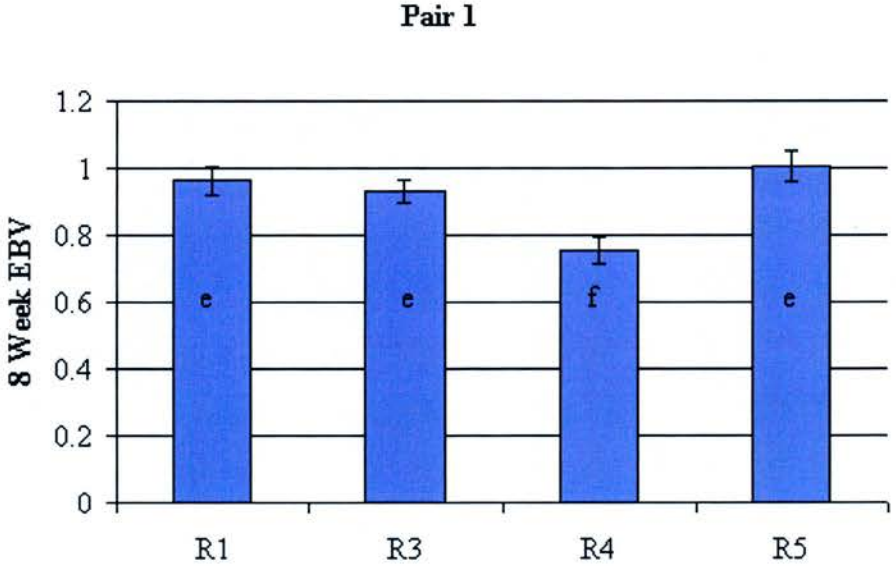

D34
Index Score
160
140
120
100
80
60
40
20
0
e
e
f
f
R1
R3
R4
R5

Paired Farms

xii

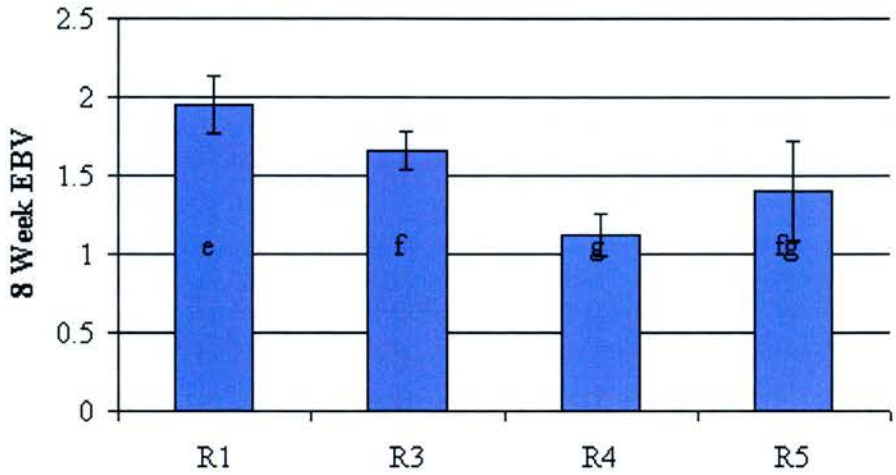


xiii



xiv

Pair 3



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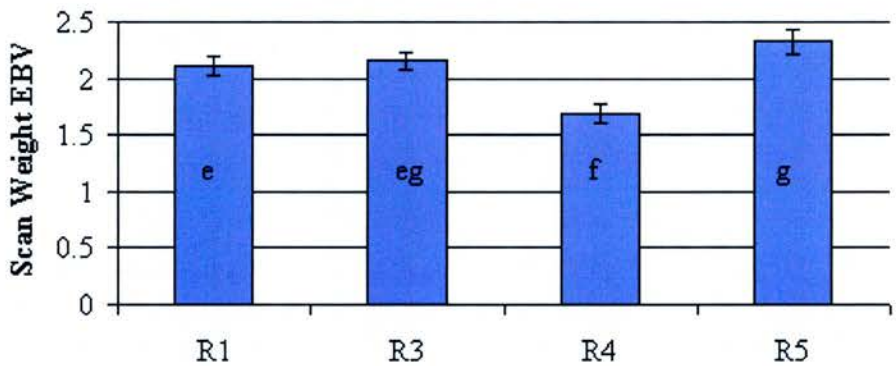

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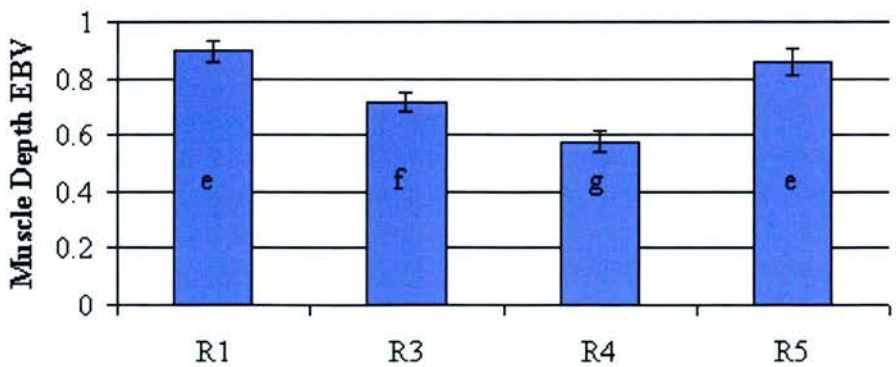
Pair 1



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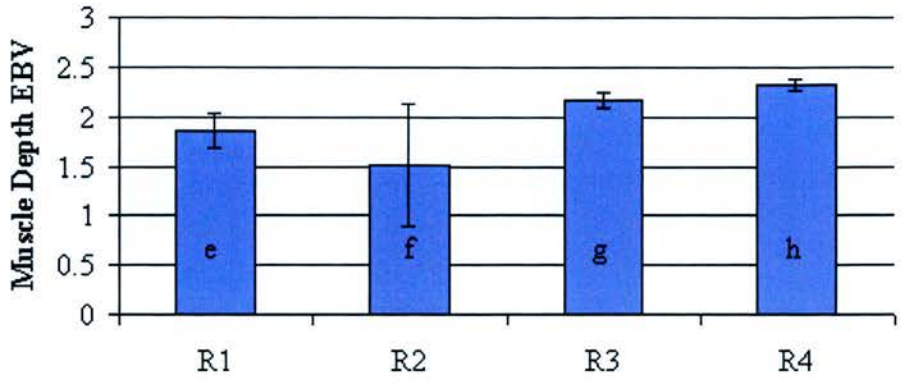

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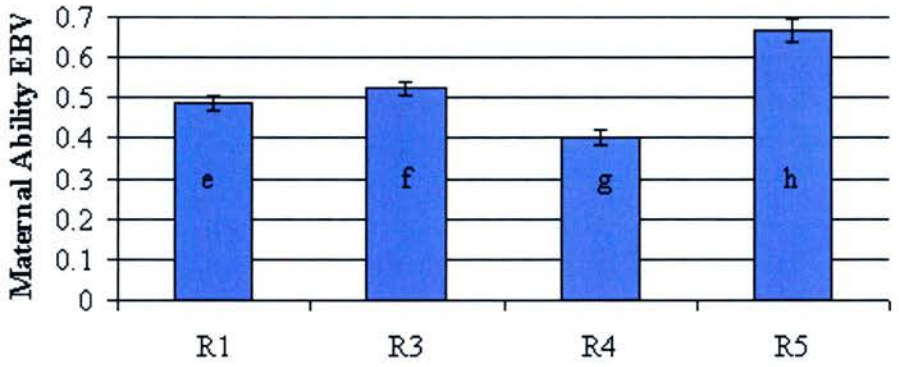
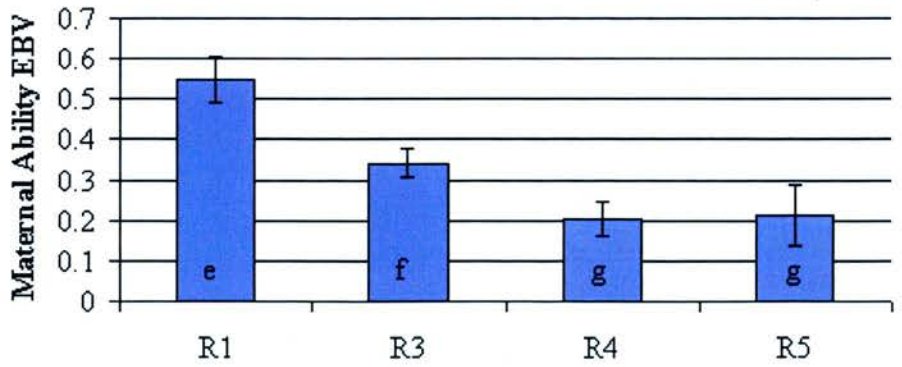
Pair 1

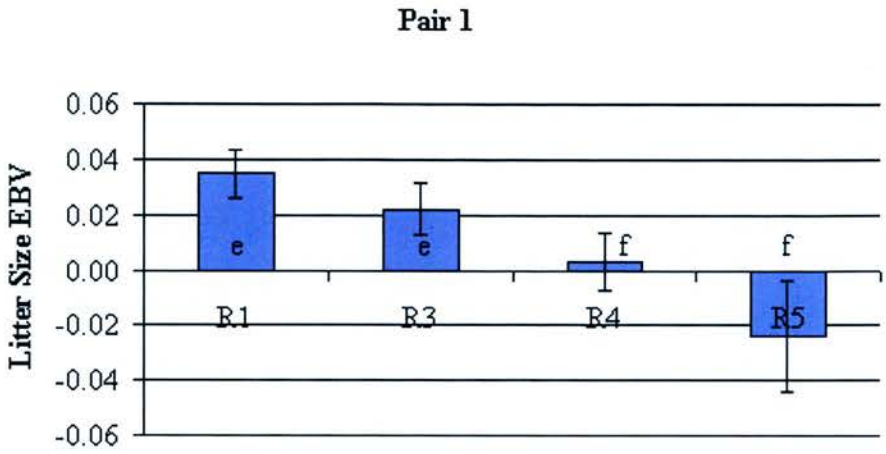


xix

Pair 3



xx



3.1.2 Figure 2 – Allelic Group

Individual Farms

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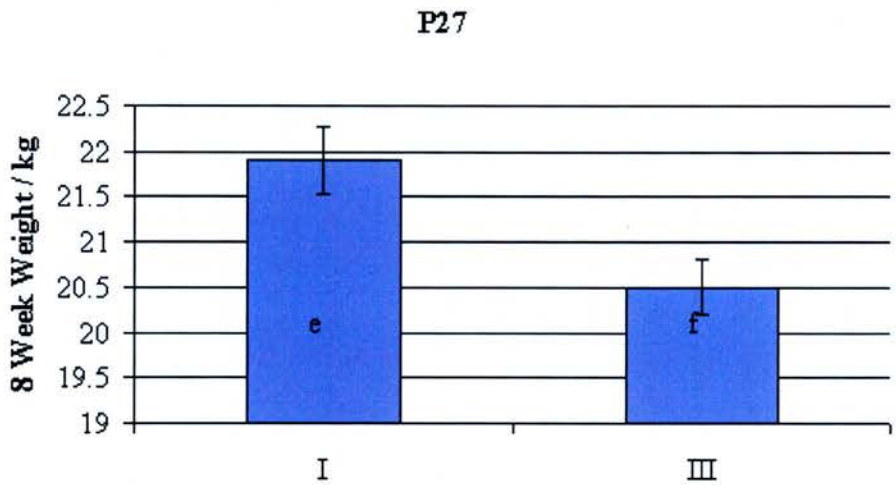

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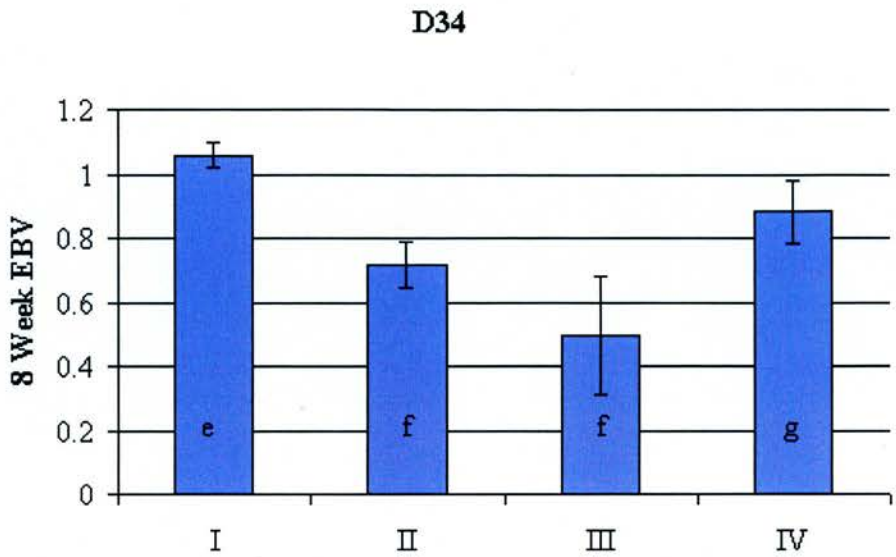
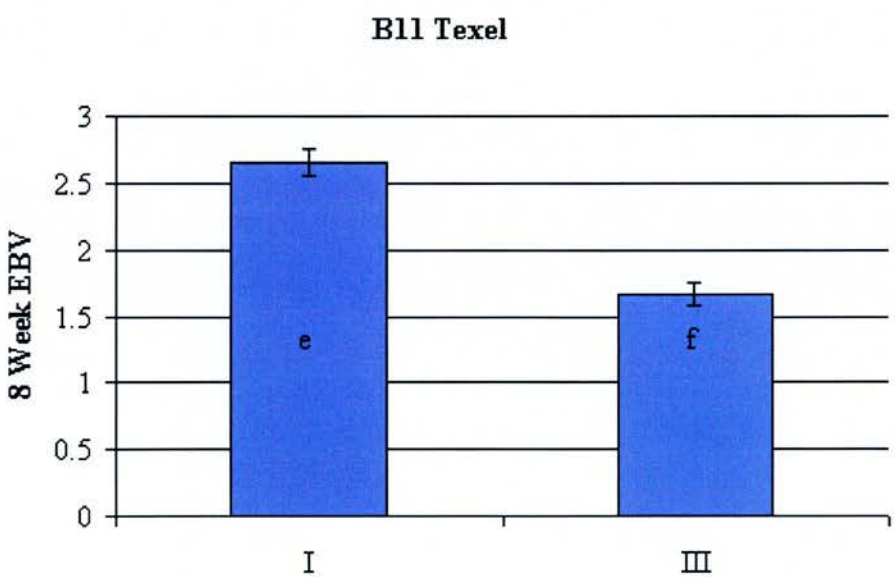
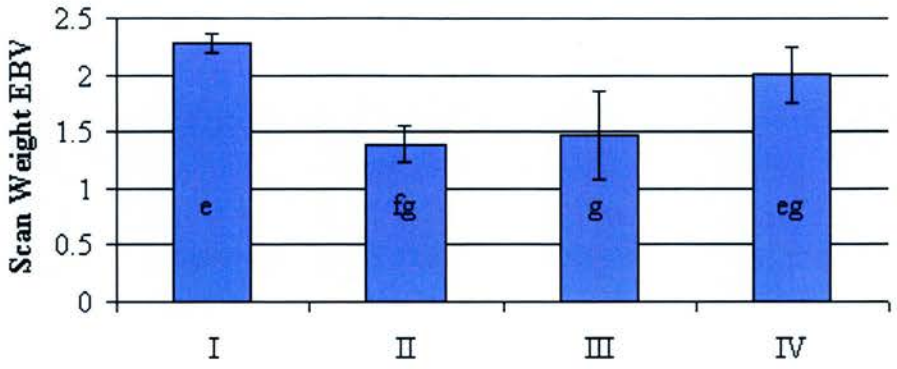

D34
8 Week EBV
1.2
1
0.8
0.6
0.4
0.2
0
e
f
f
g
I
II
III
IV

iii


B11 Texel
8 Week EBV
3
2.5
2
1.5
1
0.5
0
e
f
I
III

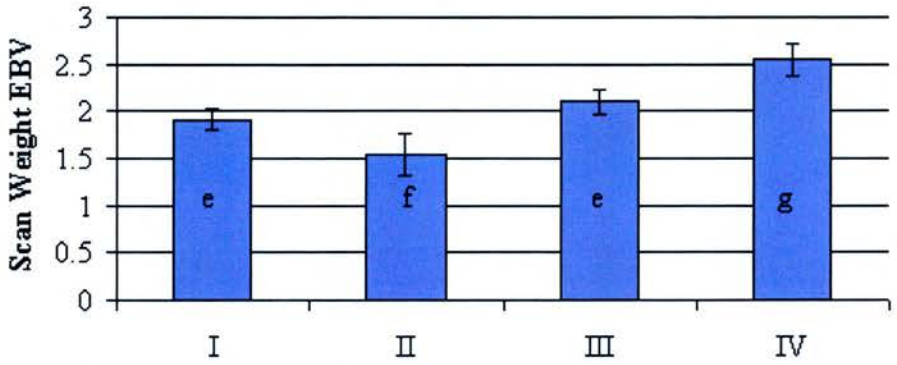
iv

D34



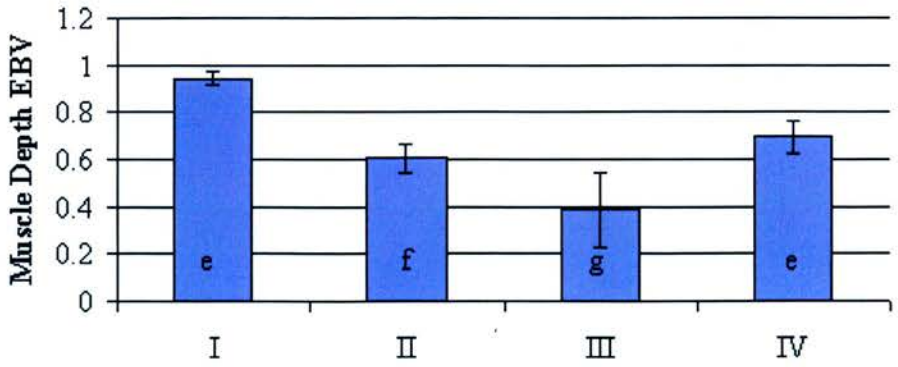
v

H19



vi

D34



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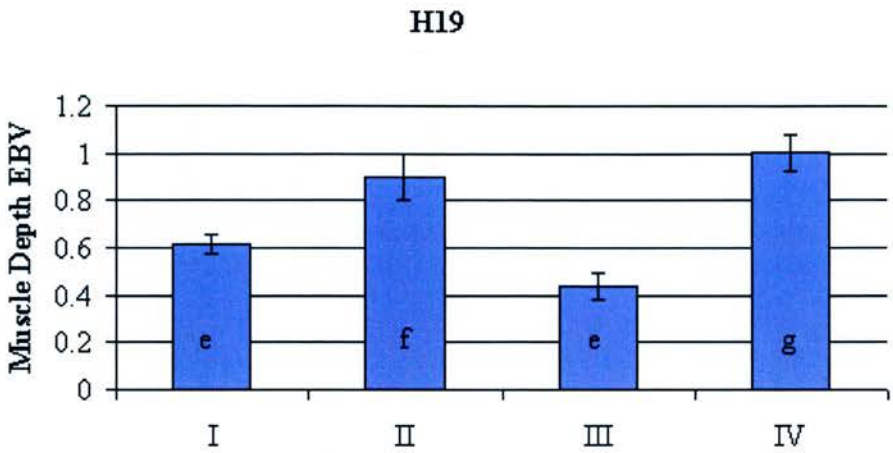
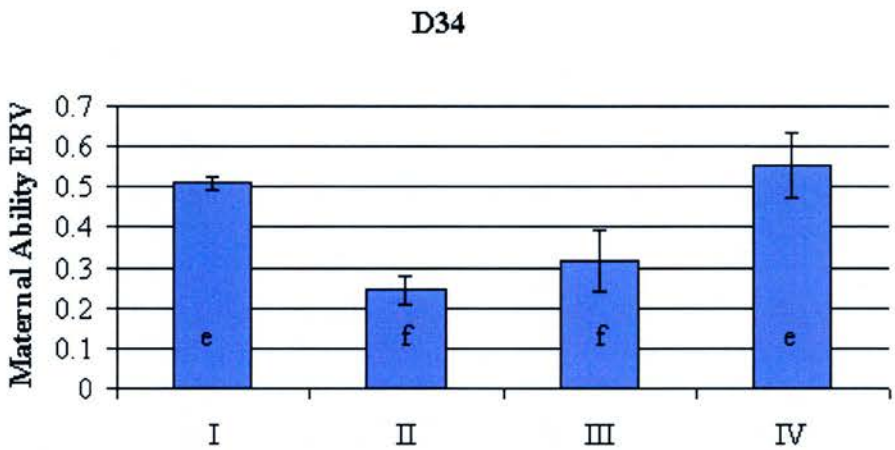
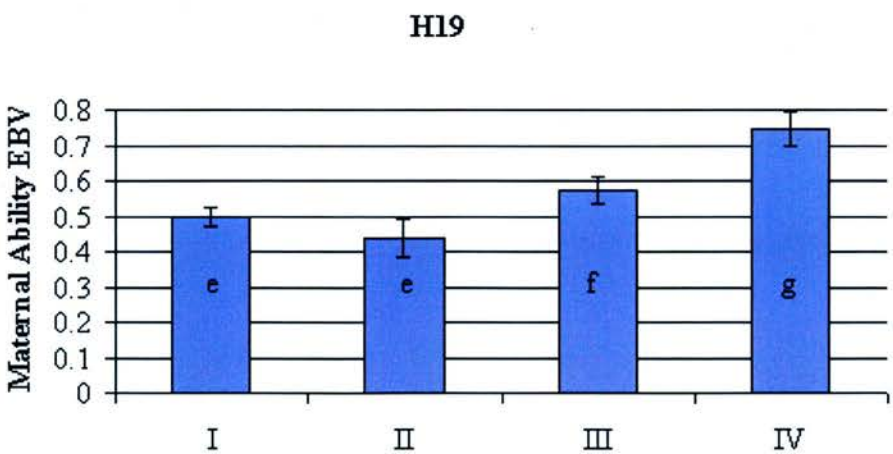

H19
Muscle Depth EBV
1.2
1
0.8
0.6
0.4
0.2
0
e
f
e
g
I
II
III
IV

viii


D34
Maternal Ability EBV
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
e
f
f
e
I
II
III
IV

ix


H19
Maternal Ability EBV
0.8
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0.6
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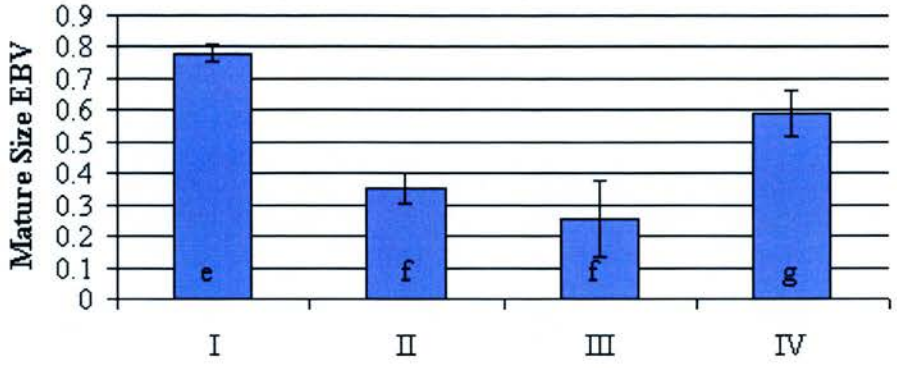
0.4
0.3
0.2
0.1
0
e
e
f
g
I
II
III
IV

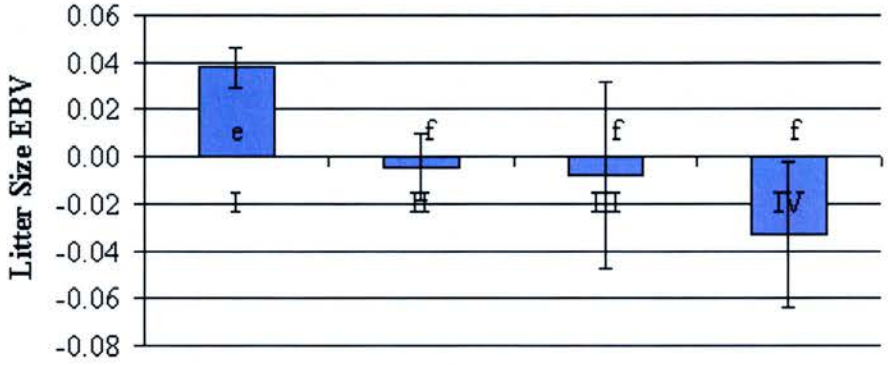
x

D34



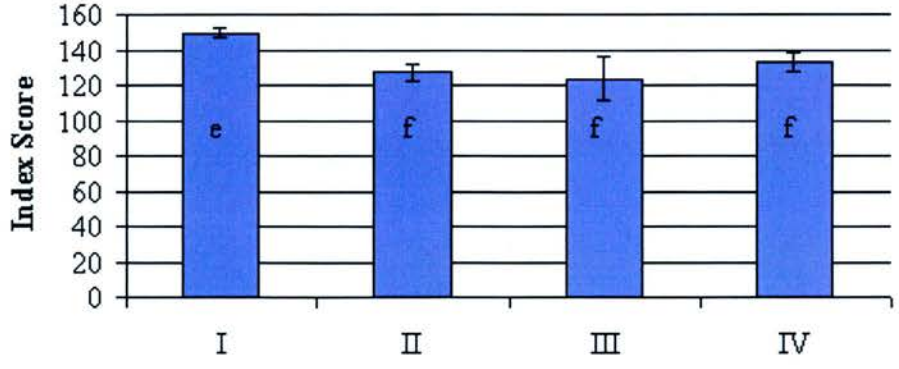
xi

D34



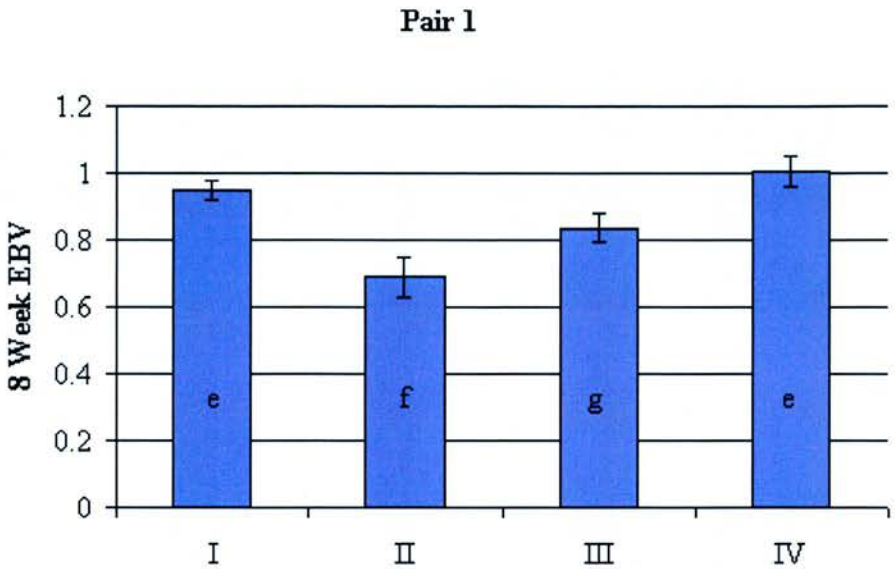
xii

D34

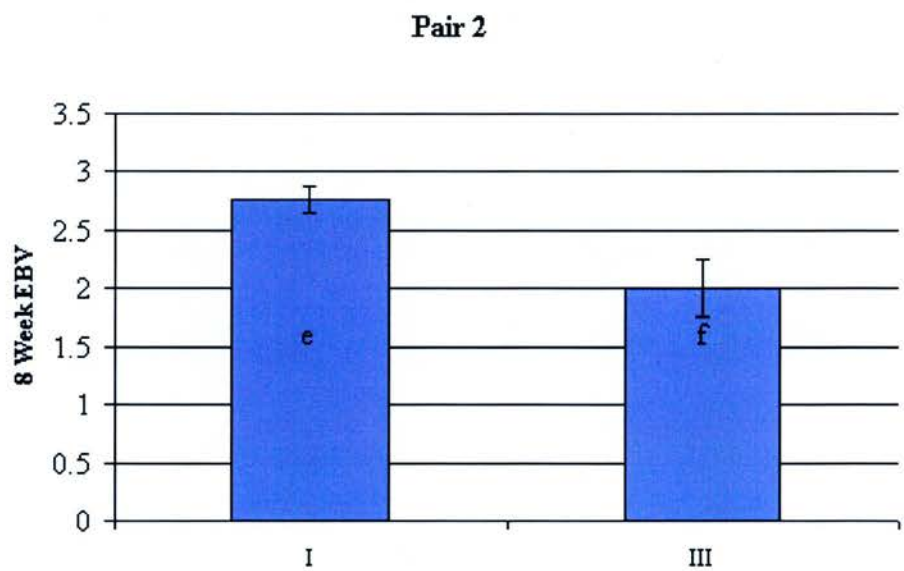


Paired Farms

xiii

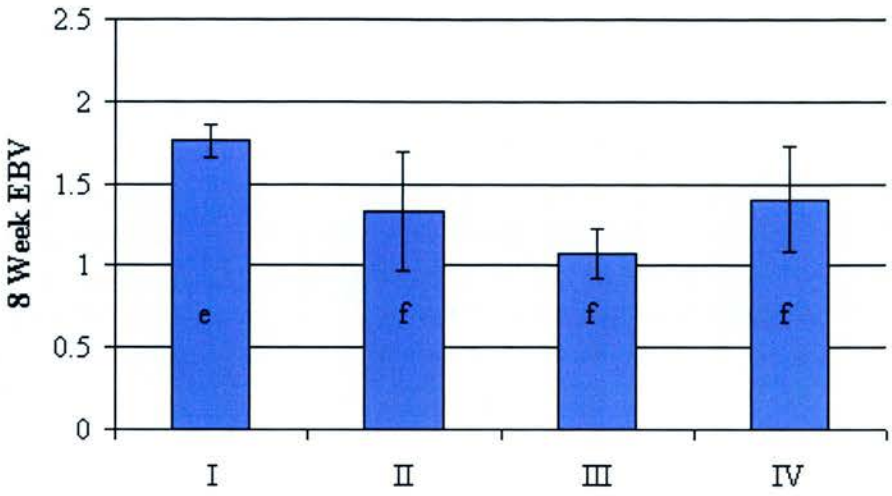


xiv



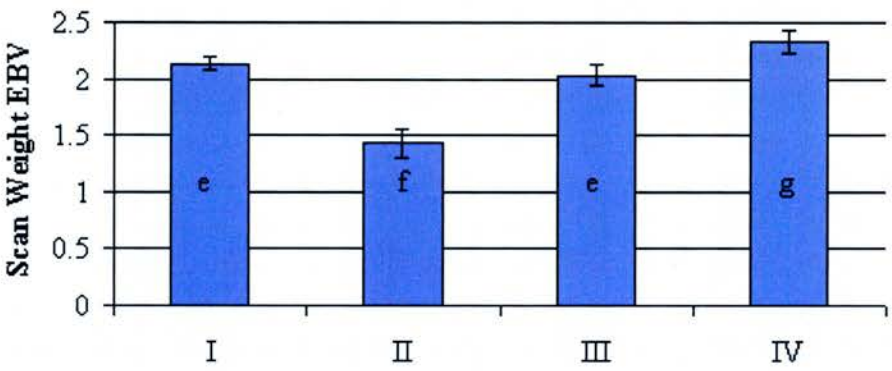
xv

Pair 3



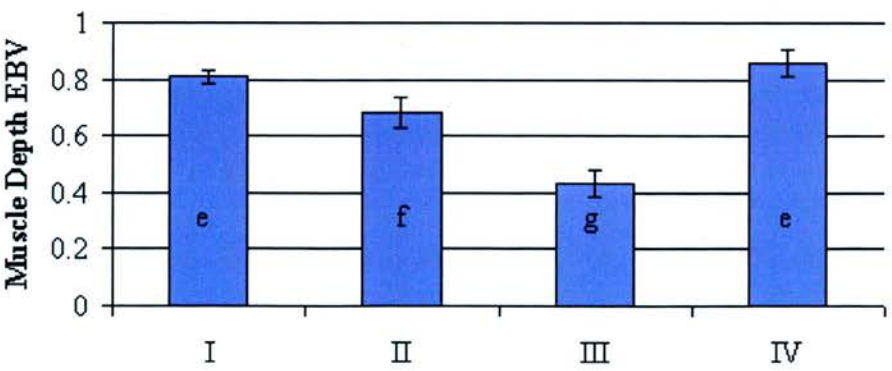
xvi

Pair 1

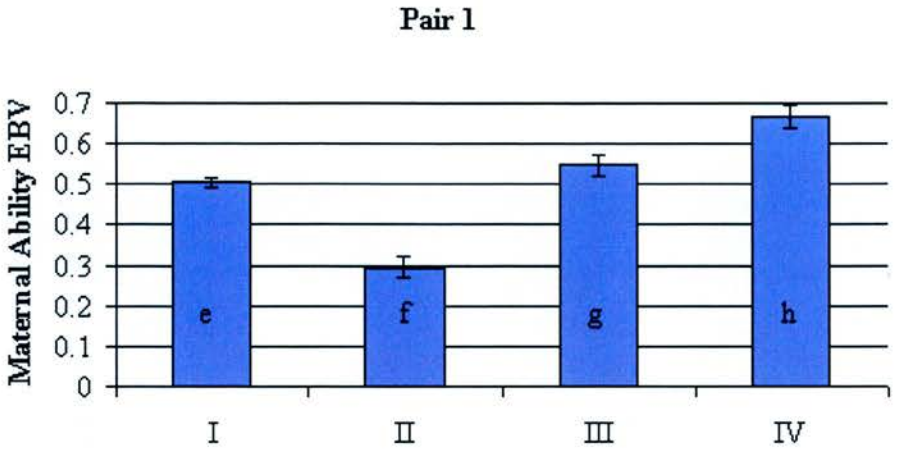


xvii

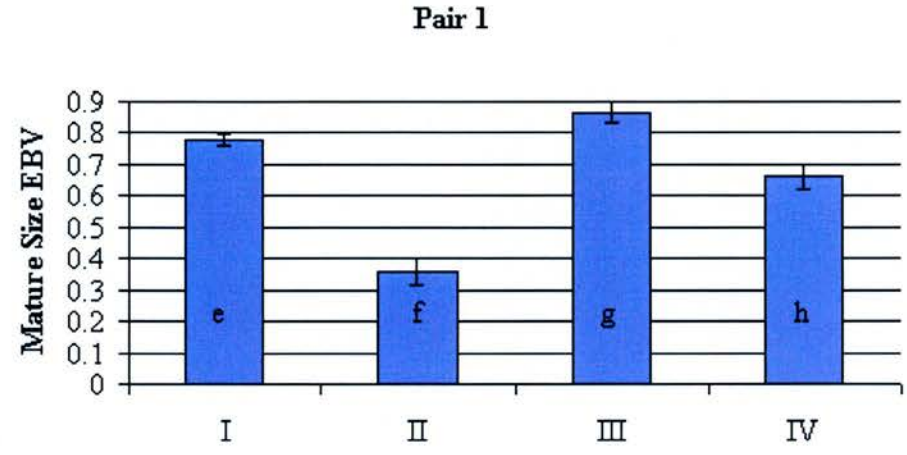
Pair 1



xviii


Pair 1
Maternal Ability EBV
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
e
f
g
h
I
II
III
IV

xix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0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
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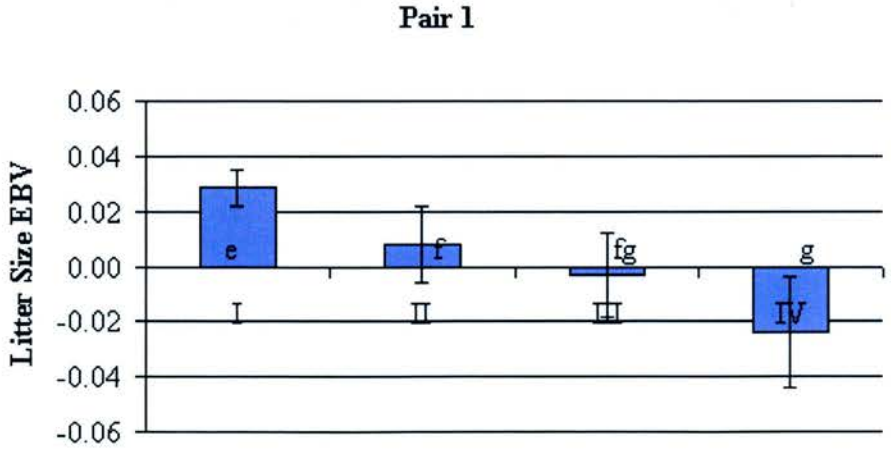
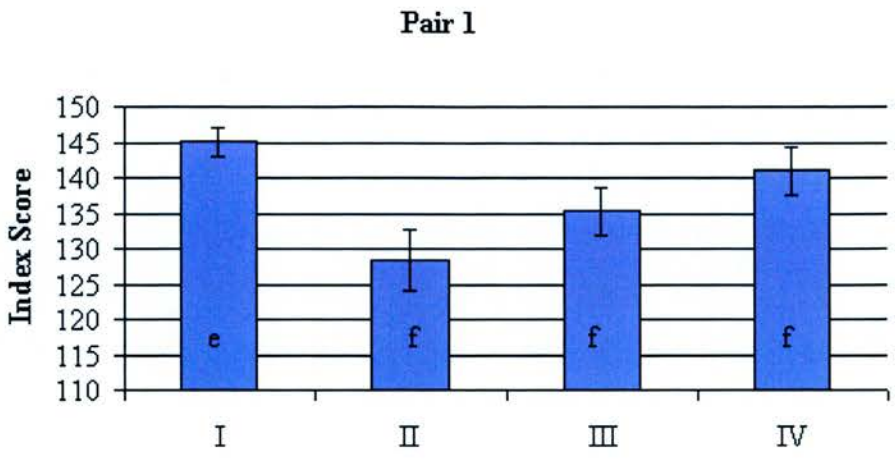
f
g
h
I
II
III
IV

xx


Pair 1
Litter Size EBV
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
e
f
fg
g
I
II
III
IV

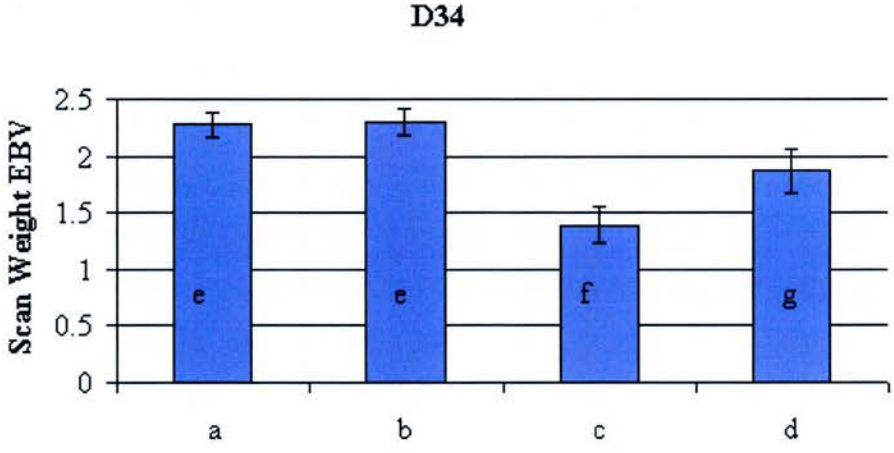
xxi



3.1.3 Figure 3 – ERA Group

Individual Farms

i



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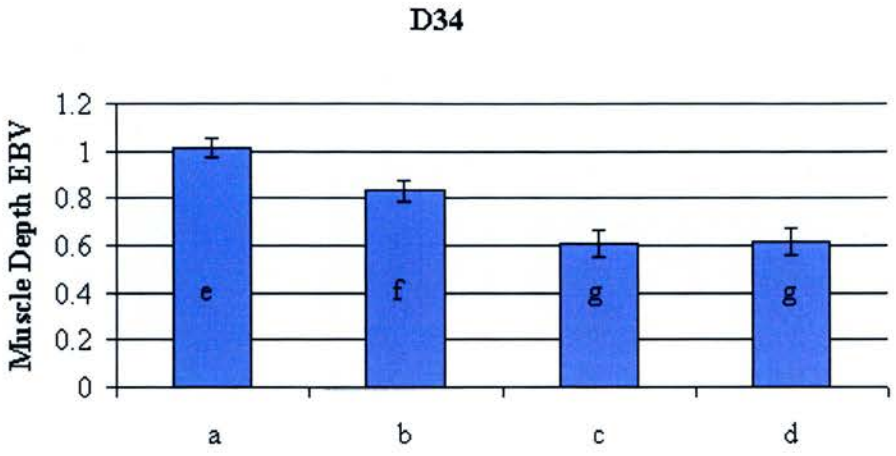
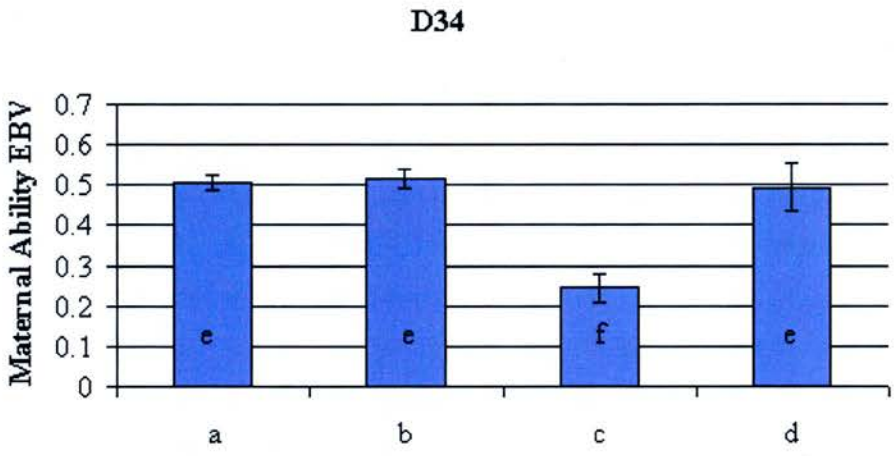
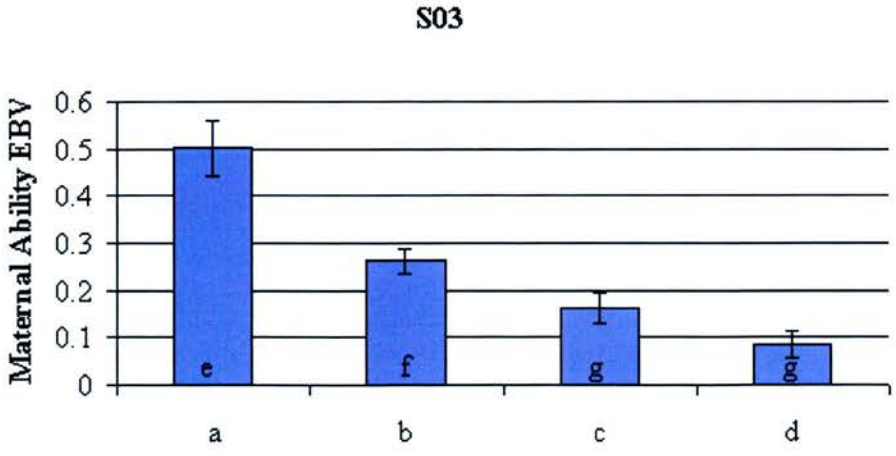

D34
Muscle Depth EBV
1.2
1
0.8
0.6
0.4
0.2
0
e
f
g
g
a
b
c
d

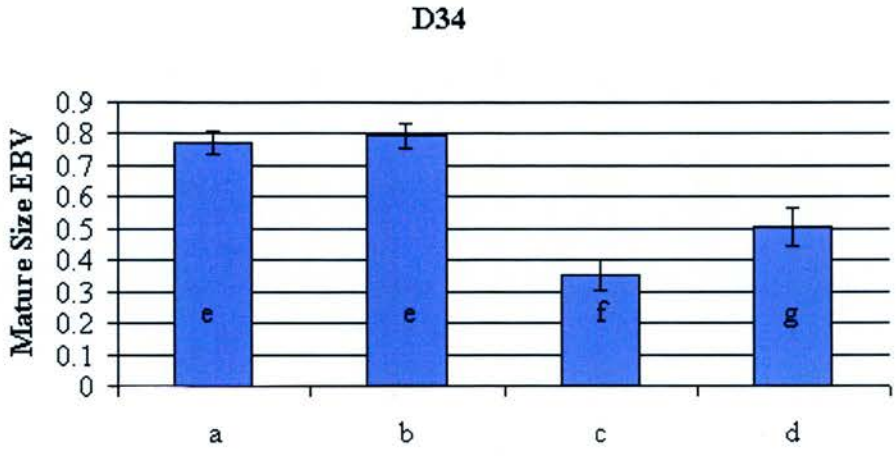
iii


D34
Maternal Ability EBV
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
e
e
f
e
a
b
c
d

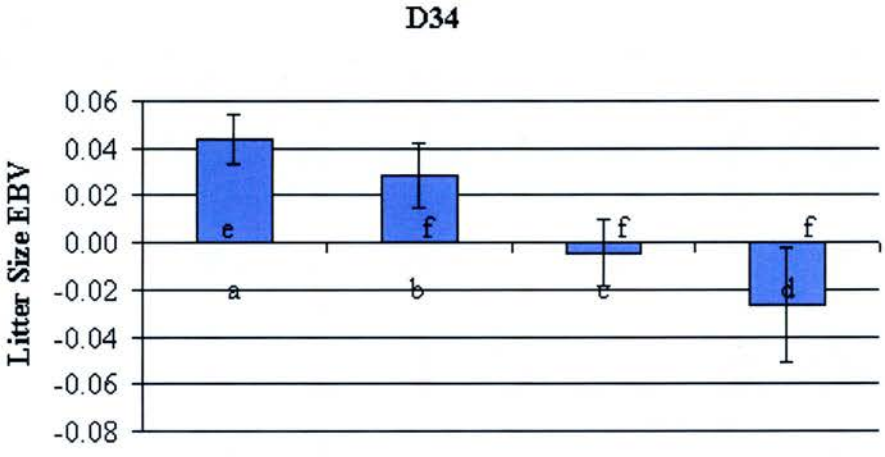
iv


S03
Maternal Ability EBV
0.6
0.5
0.4
0.3
0.2
0.1
0
e
f
g
g
a
b
c
d

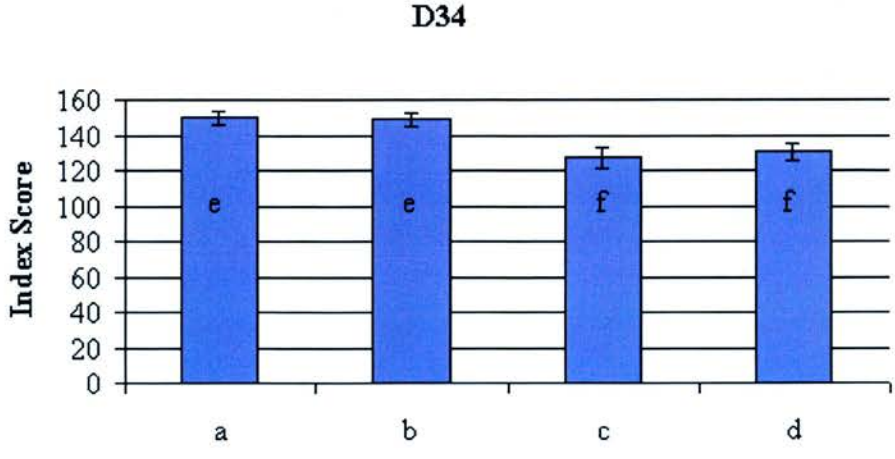
v


D34
Mature Size EBV
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
e
e
f
g
a
b
c
d

vi

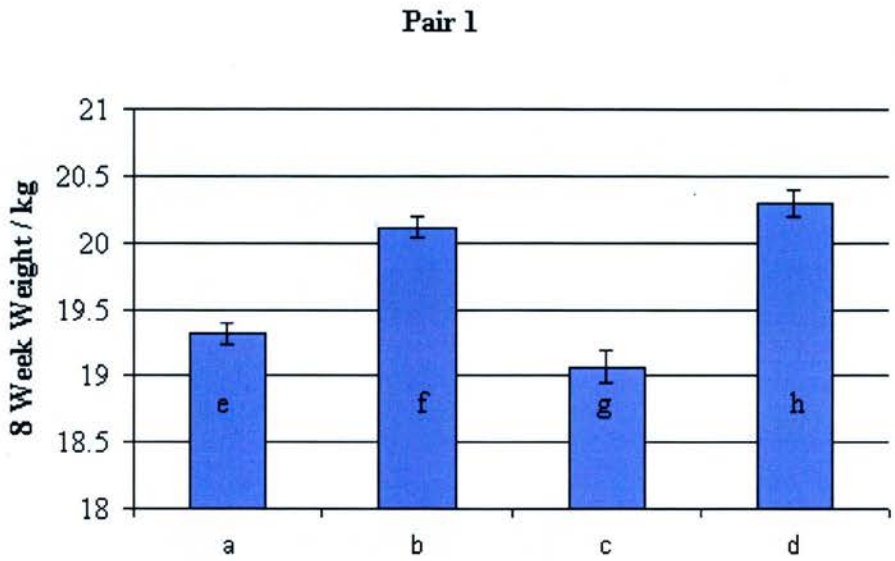

D34
Litter Size EBV
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
e
f
f
f
a
b
c
d

vii

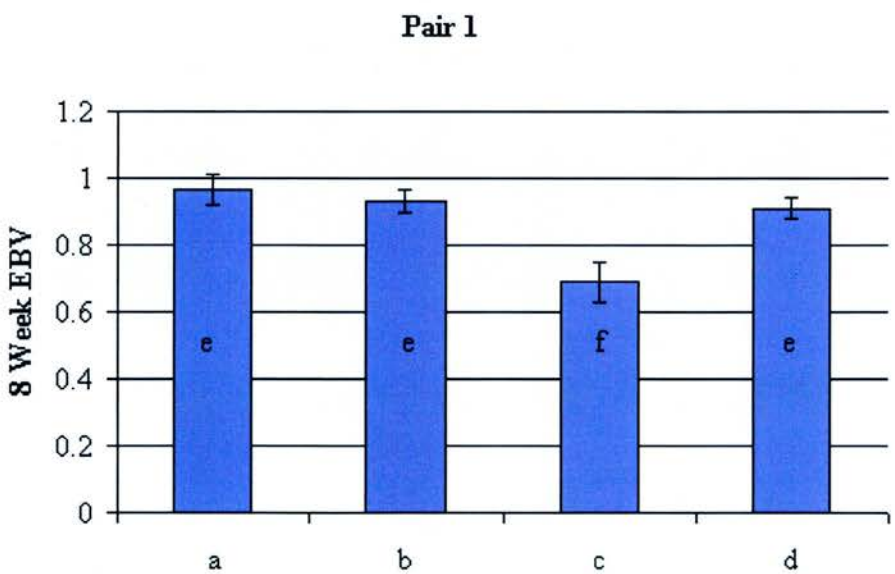

D34
Index Score
160
140
120
100
80
60
40
20
0
e
e
f
f
a
b
c
d

Paired Farms

viii

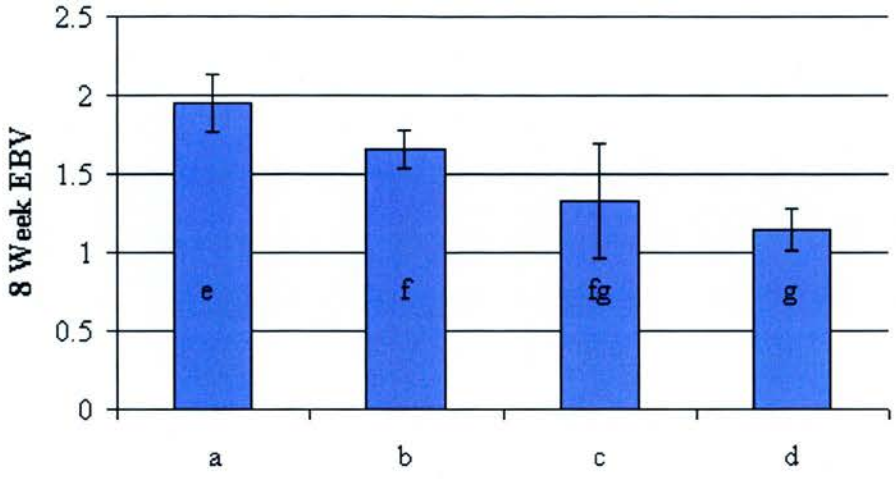


ix



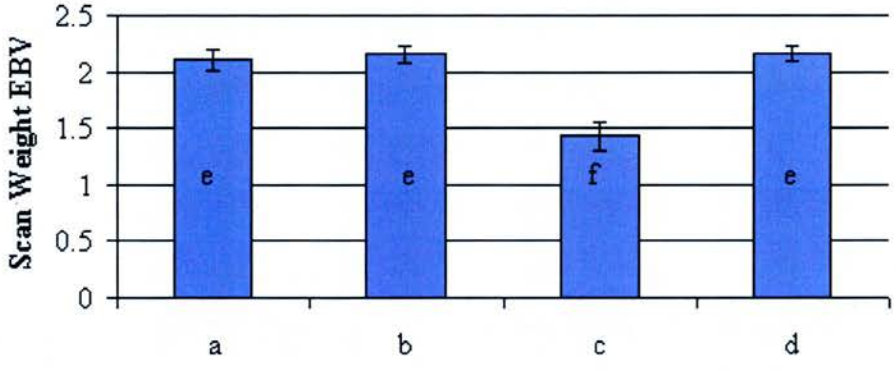
x

Pair 3



xi

Pair 1



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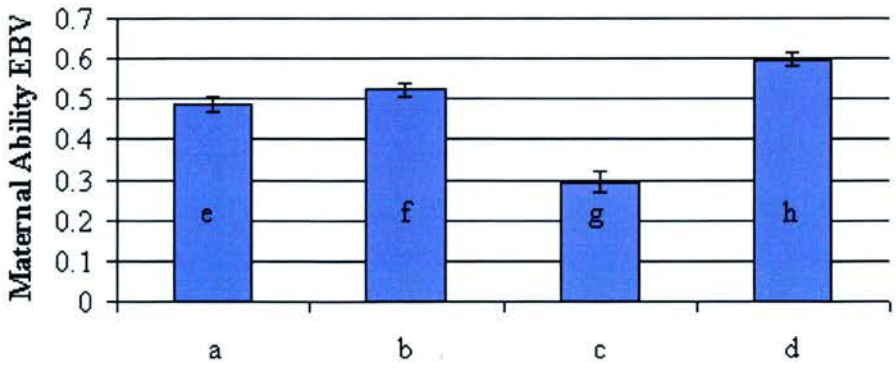
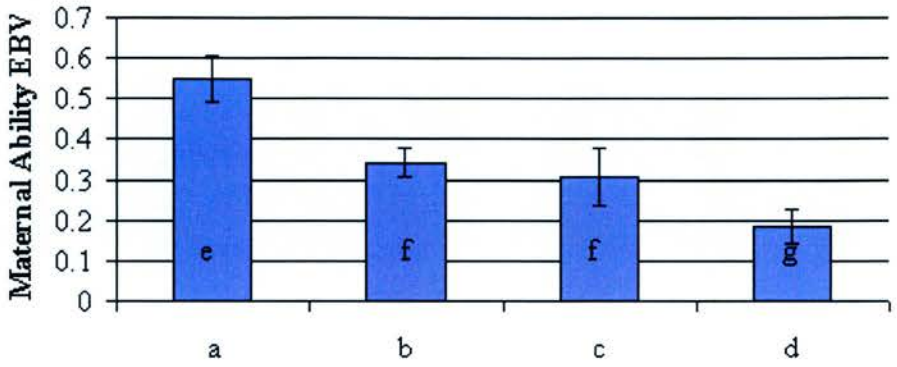
Pair 1



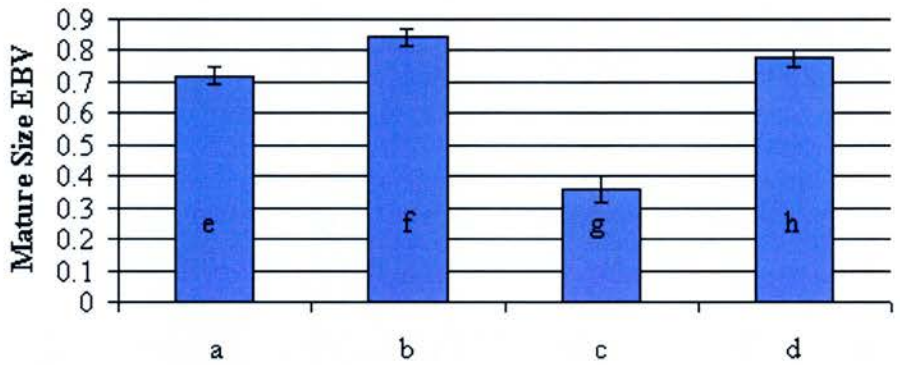
xiii

Pair 3



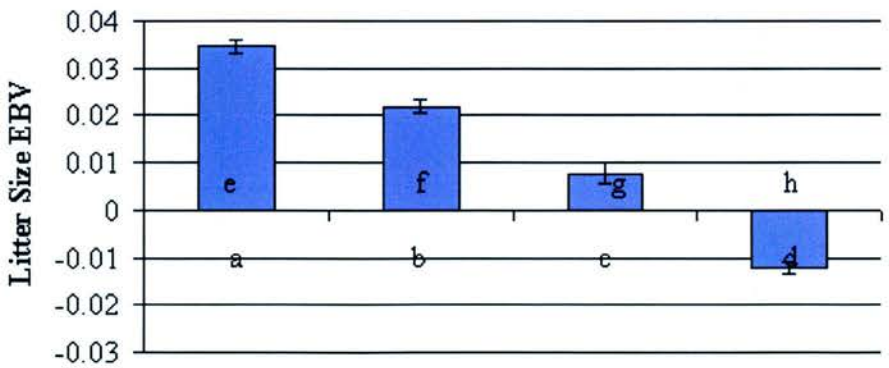
xiv

Pair 1

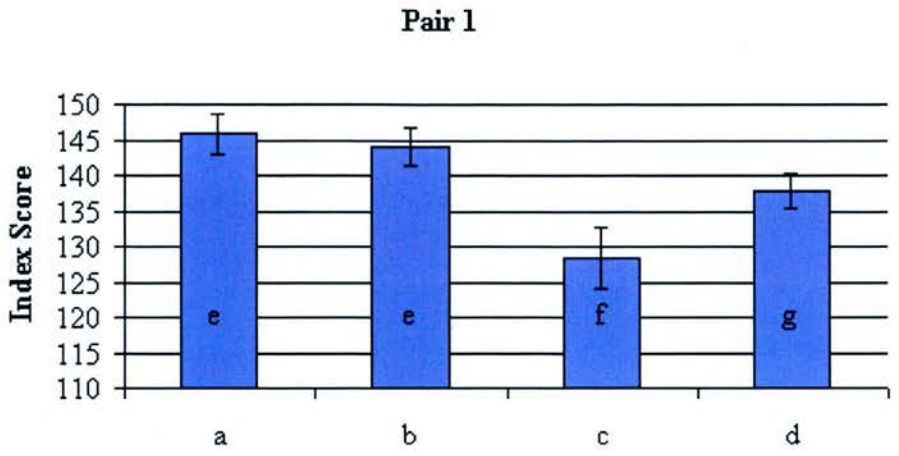


xv

Pair 1



xvi



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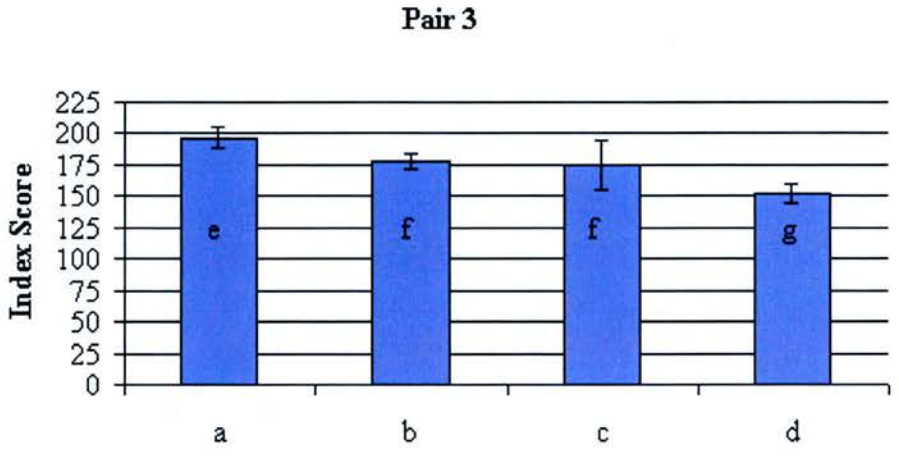

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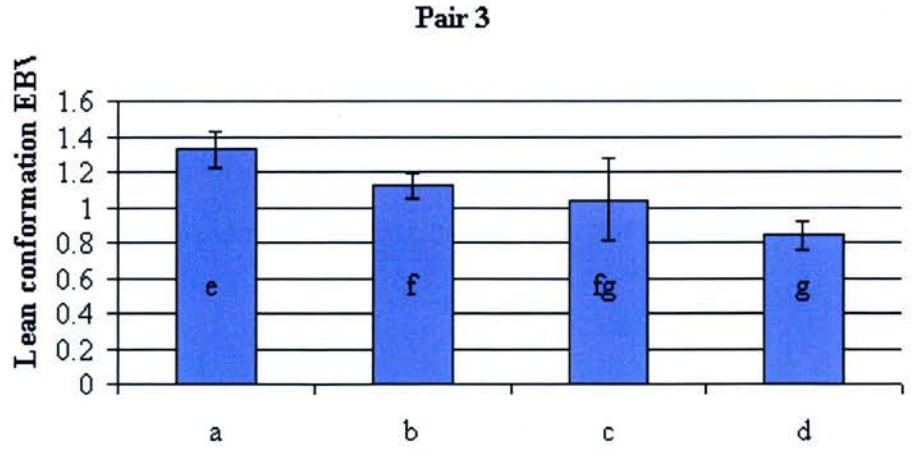

3.2 Tables of the differences between the genotypes of means for each of the Signet traits.

The follow tables indicate the differences between the means for each genotype for each of the Signet traits, and the standard error of that difference. Individual farms are listed first, then the paired farms. Means which are significantly different are highlighted in bold. SE – standard error

3.2.1 Risk group

Trait	Farm	Risk group	Mean	Difference in means			SE of difference		
8-week EBV				R3	R4	R5	R3	R4	R5
	D34	R1	1.090	**-0.083**	**-0.393**	**-0.208**	0.039	0.043	0.055
		R3	1.007		**-0.310**	**-0.125**		0.044	0.056
		R4	0.696			**0.185**			0.059
		R5	0.881						
	H19	R1	0.663	**0.204**	**0.138**	**0.428**	0.046	0.046	0.054
		R3	0.868		-0.066	**0.224**		0.035	0.046
		R4	0.801			**0.290**			0.046
		R5	1.092						
				R2	R3	R4	R2	R3	R4
	B11 Texel	R1	2.544	0.301	0.123	**-1.042**	0.180	0.148	0.143
		R2	2.845		-0.178	**-1.343**		0.115	0.109
		R3	2.667			**-1.165**			0.038
		R4	1.502						
Scan weight EBV				R3	R4	R5	R3	R4	R5
	D34	R1	2.277	0.020	**-0.880**	-0.272	0.083	0.093	0.132
		R3	2.297		**-0.900**	-0.292		0.097	0.135
		R4	1.397			0.609			0.141
		R5	2.006						
Muscle depth EBV				R3	R4	R5	R3	R4	R5
	D34	R1	1.013	**-0.181**	**-0.428**	**-0.317**	0.032	0.035	0.040
		R3	0.832		**-0.247**	**-0.136**		0.036	0.041
		R4	0.585			0.111			0.043
		R5	0.696						

3.2.1 Risk group (cont.)

Trait	Farm	Risk group	Mean	Difference in means			SE of difference		
Maternal ability EBV				R3	R4	R5	R3	R4	R5
	D34	R1	0.506	0.010	**-0.254**	0.048	0.016	0.019	0.040
		R3	0.516		**-0.265**	0.037		0.020	0.040
		R4	0.251			0.302			0.042
		R5	0.553						
	H19	R1	0.444	**0.086**	**0.090**	**0.302**	0.029	0.028	0.034
		R3	0.529		0.005	**0.216**		0.023	0.029
		R4	0.534			**0.211**			0.029
		R5	0.745						
	S03	R1	0.501	**-0.238**	**-0.411**	**-0.356**	0.032	0.032	0.032
		R3	0.263		**-0.173**	**-0.118**		0.019	0.020
		R4	0.090			0.055			0.021
		R5	0.145						
Mature size EBV				R3	R4	R5	R3	R4	R5
	D34	R1	0.770	0.025	**-0.426**	**-0.181**	0.027	0.030	0.041
		R3	0.795		**-0.451**	**-0.205**		0.031	0.041
		R4	0.344			**0.246**			0.044
		R5	0.590						
Litter size EBV				R3	R4	R5	R3	R4	R5
	D34	R1	0.044	-0.015	**-0.049**	**-0.076**	0.009	0.008	0.016
		R3	0.028		**-0.033**	**-0.061**		0.010	0.017
		R4	-0.005			-0.028			0.016
		R5	-0.033						
Index score				R3	R4	R5	R3	R4	R5
	D34	R1	149.946	-1.172	**-23.257**	**-17.256**	2.708	3.096	3.243
		R3	148.774		**-22.085**	**-16.084**		3.263	3.403
		R4	126.689			6.000			3.719
		R5	132.690						
8-week weight/ kg				R3	R4	R5	R3	R4	R5
	Pair 1	R1	19.317	**0.796**	0.150	**1.390**	0.059	0.066	0.091
		R3	20.114		**-0.646**	**0.594**		0.064	0.090
		R4	19.468			**1.240**			0.094
		R5	20.708						

3.2.1 Risk group (cont.)

Trait	Farm	Risk group	Mean	Difference in means			SE of difference		
8-week EBV				R3	R4	R5	R3	R4	R5
	Pair 1	R1	0.965	-0.034	**-0.213**	0.041	0.028	0.030	0.031
		R3	0.932		**-0.179**	0.074		0.027	0.028
		R4	0.752			0.254			0.030
		R5	1.006						
	Pair 3	R1	1.954	**-0.296**	**-0.828**	**-0.549**	0.110	0.114	0.178
		R3	1.658		**-0.532**	-0.253		0.091	0.163
		R4	1.126			0.279			0.167
		R5	1.405						
Scan weight EBV				R3	R4	R5	R3	R4	R5
	Pair 1	R1	2.111	0.043	**-0.421**	**0.215**	0.061	0.063	0.070
		R3	2.154		**-0.464**	0.172		0.060	0.067
		R4	1.690			**0.636**			0.069
		R5	2.326						
Muscle depth EBV				R3	R4	R5	R3	R4	R5
	Pair 1	R1	0.897	**-0.183**	**-0.320**	-0.038	0.025	0.027	0.030
		R3	0.715		**-0.137**	**0.145**		0.026	0.029
		R4	0.577			**0.282**			0.031
		R5	0.859						
	Pair 2	R1	1.863	**-0.345**	**0.299**	**0.464**	0.256	0.094	0.091
		R2	1.519		**0.644**	**0.809**		0.244	0.243
		R3	2.162			**0.166**			0.049
		R4	2.328						
Maternal ability EBV				R3	R4	R5	R3	R4	R5
	Pair 1	R1	0.487	**0.036**	**-0.086**	**0.179**	0.012	0.013	0.016
		R3	0.523		**-0.122**	**0.144**		0.012	0.015
		R4	0.401			**0.265**			0.016
		R5	0.667						
	Pair 3	R1	0.549	**-0.207**	**-0.345**	**-0.336**	0.034	0.036	0.046
		R3	0.342		**-0.138**	**-0.129**		0.029	0.040
		R4	0.204			0.010			0.042
		R5	0.214						

3.2.1 Risk group (cont.)

Trait	Farm	Risk group	Mean	Difference in means			SE of difference		
Litter size EBV				R3	R4	R5	R3	R4	R5
	Pair 1	R1	0.035	-0.013	**-0.032**	**-0.058**	0.007	0.007	0.011
		R3	0.022		**-0.019**	**-0.046**		0.007	0.011
		R4	0.003			-0.027			0.011
		R5	-0.024						

3.2.2 Allelic group

Trait	Farm	Allelic group	Mean	Difference in means			SE of difference		
8-week weight / kg				II	III	IV	II	III	IV
	P27	I	21.903		**0.241**			-1.398	
		III	20.506						
8-week EBV				II	III	IV	II	III	IV
	D34	I	1.058	**-0.341**	**-0.561**	**-0.177**	0.041	0.085	0.052
		II	0.718		-0.221	**0.164**		0.090	0.060
		III	0.497			**0.384**			0.095
		IV	0.881						
	B11 Texel	I	2.655		**-0.993**			0.063	
		III	1.662						
Scan weight EBV				II	III	IV	II	III	IV
	D34	I	2.285	-0.896	-0.812	-0.279	0.089	0.177	0.125
		II	1.389		0.084	0.617		0.189	0.142
		III	1.473			0.533			0.208
		IV	2.006						
	H19	I	1.915	**-0.378**	0.185	**0.632**	0.120	0.083	0.103
		II	1.536		**0.563**	**1.011**		0.126	0.140
		III	2.099			**0.448**			0.110
		IV	2.547						

3.2.2 Allelic group (cont.)

Trait	Farm	Allelic group	Mean	Difference in means			SE of difference		
Muscle depth EBV				II	III	IV	II	III	IV
	D34	I	0.944	-0.338	-0.557	-0.249	0.035	0.072	0.038
		II	0.606		-0.219	0.090		0.076	0.046
		III	0.387			0.309			0.078
		IV	0.696						
	H19	I	0.616	**0.284**	**-0.176**	0.387	0.052	0.036	0.043
		II	0.900		-0.460	**0.103**		0.055	0.060
		III	0.440			**0.563**			0.047
		IV	1.003						
Maternal ability EBV				II	III	IV	II	III	IV
	D34	I	0.509	-0.265	-0.191	0.044	0.019	0.035	0.039
		II	0.244		0.074	0.309		0.038	0.042
		III	0.318			0.235			0.051
		IV	0.553						
	H19	I	0.498	-0.062	**0.076**	**0.247**	0.030	0.023	0.028
		II	0.436		**0.137**	**0.309**		0.033	0.036
		III	0.574			**0.172**			0.031
		IV	0.745						
Mature size EBV				II	III	IV	II	III	IV
	D34	I	0.780	**-0.426**	**-0.525**	**-0.190**	0.028	0.056	0.039
		II	0.353		-0.098	**0.236**		0.060	0.044
		III	0.255			**0.335**			0.066
		IV	0.590						
Litter size EBV				II	III	IV	II	III	IV
	D34	I	0.038	**-0.042**	**-0.046**	**-0.071**	0.008	0.018	0.016
		II	-0.004		-0.004	-0.028		0.019	0.017
		III	-0.008			-0.025			0.023
		IV	-0.033						
Index score				II	III	IV	II	III	IV
	D34	I	149.503	**-22.470**	**-26.003**	**-16.813**	3.009	5.709	2.982
		II	127.032		-3.532	5.657		6.166	3.785
		III	123.500			9.190			6.153
		IV	132.690						

3.2.2 Allelic group (cont.)

Trait	Farm	Allelic group	Mean	Difference in means			SE of difference		
8-week EBV				II	III	IV	II	III	IV
	Pair 1	I	0.949	**-0.258**	**-0.113**	0.057	0.034	0.026	0.026
		II	0.691		**0.146**	**0.315**		0.038	0.038
		III	0.836			**0.170**			0.031
		IV	1.006						
	Pair 2	I	2.764		**-0.758**			0.132	
		III	2.006						
	Pair 3	I	1.761	**-0.432**	**-0.687**	**-0.357**	0.178	0.091	0.161
		II	1.329		-0.255	0.075		0.187	0.229
		III	1.075			0.330			0.171
		IV	1.405						
Scan weight EBV				II	III	IV	II	III	IV
	Pair 1	I	2.132	**-0.704**	-0.100	**0.194**	0.073	0.058	0.059
		II	1.428		**0.604**	**0.898**		0.082	0.083
		III	2.032			**0.293**			0.070
		IV	2.326						
Muscle depth EBV				II	III	IV	II	III	IV
	Pair 1	I	0.809	**-0.126**	**-0.374**	0.051	0.031	0.027	0.027
		II	0.683		**-0.248**	**0.177**		0.037	0.037
		III	0.434			**0.425**			0.033
		IV	0.859						
Maternal ability EBV				II	III	IV	II	III	IV
	Pair 1	I	0.505	**-0.210**	**0.042**	**0.162**	0.015	0.014	0.015
		II	0.295		**0.251**	**0.372**		0.018	0.019
		III	0.546			**0.121**			0.018
		IV	0.667						
Mature size EBV				II	III	IV	II	III	IV
	Pair 1	I	0.780	**-0.420**	**0.085**	**-0.120**	0.023	0.020	0.022
		II	0.359		**0.505**	**0.301**		0.027	0.029
		III	0.865			**-0.204**			0.026
		IV	0.660						

3.2.2 Allelic group (cont.)

Trait	Farm	Allelic group	Mean	Difference in means			SE of difference		
Litter size EBV				II	III	IV	II	III	IV
	Pair 1	I	0.029	**-0.021**	**-0.032**	**-0.052**	0.008	0.008	0.011
		II	0.008		-0.011	**-0.032**		0.010	0.012
		III	-0.003			-0.021			0.013
		IV	-0.024						
Index score				II	III	IV	II	III	IV
	Pair 1	I	145.033	**-1.765**	**-17.534**	**-8.155**	1.984	2.645	1.916
		II	128.354		-15.768	-6.389		2.578	1.824
		III	135.298			9.379			2.526
		IV	140.958						

3.2.3 ERA group

Trait	Farm	ERA group	Mean	Difference in means			SE of difference		
Scan weight EBV				b	c	d	b	c	d
	D34	a	2.277	0.020	**-0.888**	**-0.408**	0.083	0.098	0.112
		b	2.297		**-0.909**	**-0.428**		0.101	0.116
		c	1.389			**0.480**			0.127
		d	1.869						
Muscle depth EBV				b	c	d	b	c	d
	D34	a	1.013	**-0.181**	**-0.407**	**-0.396**	0.031	0.037	0.037
		b	0.832		**-0.226**	**-0.215**		0.037	0.037
		c	0.606			0.010			0.042
		d	0.616						
Maternal ability EBV				b	c	d	b	c	d
	D34	a	0.506	0.010	**-0.262**	-0.013	0.016	0.020	0.031
		b	0.516		**-0.272**	-0.023		0.021	0.032
		c	0.244			0.249			0.034
		d	0.493						
	S03			b	c	d	b	c	d
		a	0.501	**-0.238**	**-0.340**	**-0.415**	0.032	0.032	0.032
		b	0.263		**-0.101**	**-0.176**		0.020	0.019
		c	0.161			-0.075			0.020
		d	0.086						

3.2.3 ERA group (cont.)

Trait	Farm	ERA group	Mean	Difference in means			SE of difference		
Mature size EBV				b	c	d	b	c	d
	D34	a	0.770	0.025	**-0.417**	**-0.267**	0.027	0.031	0.035
		b	0.795		**-0.442**	**-0.291**		0.032	0.035
		c	0.353			**0.150**			0.039
		d	0.504						
Litter size EBV				b	c	d	b	c	d
	D34	a	0.044	**-0.015**	**-0.048**	**-0.070**	0.009	0.009	0.013
		b	0.028		-0.033	-0.055		0.010	0.014
		c	-0.004			-0.022			0.014
		d	-0.026						
Index score				b	c	d	b	c	d
	D34	a	149.946	-1.172	**-22.914**	**-19.613**	2.707	3.256	3.029
		b	148.774		**-21.742**	**-18.441**		3.411	3.195
		c	127.032			3.301			3.672
		d	130.333						
8-week weight/ kg				b	c	d	b	c	d
	Pair 1	a	19.317	**0.796**	**-0.249**	**0.982**	0.058	0.076	0.064
		b	20.114		**-1.046**	**0.186**		0.075	0.063
		c	19.068			**1.231**			0.081
		d	20.299						
8-week EBV				b	c	d	b	c	d
	Pair 1	a	0.965	-0.034	**-0.275**	-0.056	0.029	0.038	0.027
		b	0.932		**-0.241**	-0.022		0.036	0.024
		c	0.691			0.219			0.034
		d	0.909						
	Pair 3	a	1.954	**-0.296**	**-0.624**	**-0.804**	0.110	0.193	0.114
		b	1.658		-0.328	**-0.508**		0.180	0.090
		c	1.329			-0.180			0.183
		d	1.150						
Scan weight EBV				b	c	d	b	c	d
	Pair 1	a	2.111	0.043	**-0.683**	0.047	0.062	0.082	0.059
		b	2.154		**-0.726**	0.004		0.078	0.054
		c	1.428			0.731			0.075
		d	2.159						

3.2.3 ERA group (cont.)

Trait	Farm	ERA group	Mean	Difference in means			SE of difference		
Maternal ability EBV				b	c	d	b	c	d
	Pair 1	a	0.487	**0.036**	**-0.192**	**0.111**	0.012	0.016	0.012
		b	0.523		**-0.228**	**0.075**		0.016	0.012
		c	0.295			**0.303**			0.016
		d	0.598						
	Pair 3	a	0.549	**-0.207**	**-0.240**	**-0.363**	0.034	0.044	0.036
		b	0.342		-0.033	**-0.157**		0.038	0.028
		c	0.309			**-0.123**			0.040
		d	0.186						
Mature size EBV				b	c	d	b	c	d
	Pair 1	a	0.720	**0.124**	**-0.360**	**0.058**	0.020	0.026	0.020
		b	0.844		**-0.484**	**-0.066**		0.025	0.019
		c	0.359			**0.418**			0.026
		d	0.777						
Litter size EBV				b	c	d	b	c	d
	Pair 1	a	0.035	**-0.013**	**-0.027**	**-0.047**	0.001	0.001	0.001
		b	0.022		**-0.014**	**-0.034**		0.001	0.001
		c	0.008			**-0.020**			0.001
		d	-0.012						
Index				b	c	d	b	c	d
	Pair 1	a	145.888	-1.765	**-17.534**	**-8.155**	1.984	2.645	1.916
		b	144.123		**-15.768**	**-6.389**		2.578	1.824
		c	128.354			**9.379**			2.526
		d	137.733						
	Pair 3	a	196.486	**-19.270**	**-21.309**	**-44.327**	5.213	10.271	5.546
		b	177.215		-2.039	**-25.056**		9.842	4.705
		c	175.176			**-23.017**			10.022
		d	152.159						
Lean conformation EBV				b	c	d	b	c	d
	Pair 3	a	1.326	**-0.205**	**-0.283**	**-0.486**	0.063	0.121	0.066
		b	1.121		-0.078	**-0.281**		0.115	0.056
		c	1.043			-0.203			0.117
		d	0.840						

Appendix 4 Analysis of Variance Tables

Terms added sequentially (first to last). All analyses refer to data from ewes, unless otherwise stated

Individual Farms

8-week weight

Farm P27

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	9	617.6	68.6	6.43	<0.001
Allelic group	1	89.4	89.4	8.38	0.004
Residuals	197	2103.5	10.7		

8-week weight EBV

Farm D34

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	10	74.4	7.4	12.21	<0.001
Risk group	3	8.0	2.7	4.40	0.005
Residuals	505	307.8	0.6		
YoB	10	74.4	7.4	12.21	<0.001
Allelic group	3	8.1	2.7	4.42	0.004
Residuals	505	307.8	0.6		

Farm H19

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	8	42.3	5.3	7.82	<0.001
Risk group	3	7.9	2.6	3.87	0.009
Residuals	419	283.2	0.7		

B11 Texel (males)

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	4	13.9	3.5	5.97	<0.001
Risk group	3	7.6	2.5	4.34	0.007
Residuals	70	40.6	0.6		
YoB	4	13.9	3.5	5.88	<0.001
Allelic group	2	6.4	3.2	5.42	0.006
Residuals	71	41.8	0.6		

Scan weight EBV

Farm D34

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	10	337.4	33.7	11.91	<0.001
Risk group	3	56.2	18.7	6.61	<0.001
Residuals	505	1431.0	2.8		
YoB	10	337.4	33.7	11.89	<0.001
Allelic group	3	54.2	18.1	6.36	<0.001
Residuals	505	1433.0	2.8		
YoB	10	337.4	33.7	11.89	<0.001
ERA group	3	54.2	18.1	6.36	<0.001
Residuals	505	1433.0	2.8		

Farm H19

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	8	236.3	29.5	8.50	<0.001
Allelic group	3	41.6	13.9	3.99	0.008
Residuals	419	1455.2	3.5		

Muscle depth EBV

Farm D34

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	10	52.4	5.2	11.42	<0.001
Risk group	3	9.5	3.2	6.89	<0.001
Residuals	505	231.6	0.5		
YoB	10	52.4	5.2	11.37	<0.001
Allelic group	3	8.5	2.8	6.12	<0.001
Residuals	505	232.7	0.5		
YoB	10	52.4	5.2	11.37	<0.001
ERA group	3	8.3	2.8	6.04	<0.001
Residuals	505	232.8	0.5		

Farm H19

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	8	44.3	5.5	6.86	<0.001
Allelic group	3	13.0	4.3	5.39	0.001
Residuals	419	338.0	0.8		

Maternal ability EBV

Farm D34

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	10	12.5	1.3	3.93	<0.001
Risk group	3	6.2	2.1	6.50	<0.001
Residuals	505	161.0	0.3		
YoB	10	12.5	1.3	3.94	<0.001
Allelic group	3	6.4	2.1	6.65	<0.001
Residuals	505	160.9	0.3		
YoB	10	12.5	1.3	3.93	<0.001
ERA group	3	6.0	2.0	6.22	<0.001
Residuals	505	161.3	0.3		

Farm H19

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	8	15.6	1.9	5.25	<0.001
Risk group	3	4.8	1.6	4.33	0.005
Residuals	419	155.5	0.4		
YoB	8	15.6	1.9	5.25	<0.001
Allelic group	3	4.9	1.6	4.42	0.004
Residuals	419	155.4	0.4		

Farm S03

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	11	6.1	0.6	4.12	<0.001
Risk group	3	2.4	0.8	5.95	0.001
Residuals	191	25.8	0.1		
YoB	11	6.1	0.6	4.12	<0.001
ERA group	3	2.4	0.8	5.92	0.001
Residuals	191	25.8	0.1		

Mature size EBV

Farm D34	Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
	YoB	10	37.2	3.7	5.05	<0.001
	Risk group	3	12.6	4.2	5.67	0.001
	Residuals	505	372.5	0.7		
	YoB	10	37.2	3.7	5.04	<0.001
	Allelic group	3	12.3	4.1	5.53	0.001
	Residuals	505	372.8	0.7		
	YoB	10	37.2	3.7	5.04	<0.001
	ERA group	3	11.7	3.9	5.29	0.001
	Residuals	505	373.3	0.7		

Litter size EBV

Farm D34	Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
	Risk group	3	0.3	0.1	14.36	<0.001
	Residuals	515	3.3	0.0		
	Allelic group	3	0.3	0.1	13.14	<0.001
	Residuals	515	3.3	0.0		
	ERA group	3	0.3	0.1	14.11	<0.001
	Residuals	515	3.3	0.0		

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Farm D34	Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
	YoB	10	361821.1	36182.1	19.71	<0.001
	Risk group	3	27749.3	9249.8	5.04	0.002
	Residuals	504	925144.2	1835.6		
	YoB	10	361821.1	36182.1	19.66	<0.001
	Allelic group	3	25175.2	8391.7	4.56	0.004
	Residuals	504	927718.4	1840.7		
	YoB	10	361821.1	36182.1	19.68	<0.001
	ERA group	3	26494.1	8831.4	4.80	0.003
	Residuals	504	926399.4	1838.1		

Paired Farms

8-week weight

Pair 1

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	10	435.5	43.5	4.85	<0.001
Farm	1	116.5	116.5	12.98	<0.001
Risk group	3	124.9	41.6	4.64	0.003
Residuals	904	8112.2	9.0		
YoB	10	435.5	43.5	4.85	<0.001
Farm	1	116.5	116.5	12.96	<0.001
ERA group	3	113.3	37.8	4.20	0.006
Residuals	904	8123.9	9.0		

8-week weight EBV

Pair 1

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	11	107.9	9.8	14.96	<0.001
Risk group	3	8.1	2.7	4.11	0.007
Residuals	935	613.0	0.7		
YoB	11	107.9	9.8	15.07	<0.001
Allelic group	3	12.4	4.1	6.34	<0.001
Residuals	935	608.7	0.7		
YoB	11	107.9	9.8	15.03	<0.001
ERA group	3	10.8	3.6	5.52	0.001
Residuals	935	610.3	0.7		

Pair 2 (males)

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	4	15.8	3.9	6.41	<0.001
Farm	1	7.7	7.7	12.55	0.001
Allelic group	2	6.3	3.1	5.12	0.008
Residuals	85	52.2	0.6		

Pair 3

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	12	159.5	13.3	14.31	<0.001
Risk group	3	11.8	3.9	4.24	0.006
Residuals	289	268.4	0.9		
YoB	12	159.5	13.3	14.30	<0.001
Allelic group	3	11.6	3.9	4.15	0.007
Residuals	289	268.7	0.9		
YoB	12	159.5	13.3	14.33	<0.001
ERA group	3	12.2	4.1	4.38	0.005
Residuals	289	268.1	0.9		

Scan weight EBV

Pair 1

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	11	491.9	44.7	13.88	<0.001
Farm	1	14.9	14.9	4.63	0.032
Risk group	3	46.5	15.5	4.81	0.002
Residuals	932	3003.8	3.2		
YoB	11	492.9	44.8	14.07	<0.001
Allelic group	3	90.1	30.0	9.43	<0.001
Residuals	935	2977.4	3.2		
YoB	11	492.9	44.8	14.07	<0.001
ERA group	3	90.6	30.2	9.48	<0.001
Residuals	935	2976.9	3.2		

Muscle depth EBV

Pair 1

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	11	94.7	8.6	13.58	<0.001
Farm	1	2.3	2.3	3.56	0.060
Risk group	3	9.9	3.3	5.21	0.001
Residuals	932	591.1	0.6		
YoB	11	94.7	8.6	13.53	<0.001
Farm	1	2.3	2.3	3.55	0.060
Allelic group	3	7.8	2.6	4.06	0.007
Residuals	932	593.2	0.6		

Pair 2

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	10	51.5	5.2	7.67	<0.001
Risk group	4	11.5	2.9	4.28	0.002
Residuals	359	241.0	0.7		

Maternal ability EBV

Pair 1

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	11	19.6	1.8	5.02	<0.001
Risk group	3	5.2	1.7	4.84	0.002
Residuals	935	332.1	0.4		
YoB	11	19.6	1.8	5.08	<0.001
Allelic group	3	8.8	2.9	8.36	<0.001
Residuals	935	328.4	0.4		
YoB	11	19.6	1.8	5.07	<0.001
ERA group	3	7.9	2.6	7.51	<0.001
Residuals	935	329.3	0.4		

Pair 3

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	12	12.8	1.1	5.38	<0.001
Scrapie	1	2.9	2.9	14.59	<0.001
Risk group	3	2.7	0.9	4.56	0.004
Residuals	288	56.9	0.2		
YoB	12	12.8	1.1	5.40	<0.001
Scrapie	1	2.9	2.9	14.64	<0.001
ERA group	3	2.9	1.0	4.91	0.002
Residuals	288	56.7	0.2		

Mature size EBV

Pair 1

Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
YoB	11	48.7	4.4	4.75	<0.001
Farm	1	8.3	8.3	8.89	0.003
Allelic group	3	17.4	5.8	6.22	<0.001
Residuals	932	868.2	0.9		
YoB	11	48.7	4.4	4.77	<0.001
Farm	1	8.3	8.3	8.92	0.003
ERA group	3	20.3	6.8	7.31	<0.001
Residuals	932	865.2	0.9		

Litter size EBV

Pair 1	Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
	Risk group	3	0.3	0.1	13.38	<0.001
	Residuals	946	6.4	0.0		
	Allelic group	3	0.3	0.1	12.35	<0.001
	Residuals	946	6.4	0.0		
	YoB	11	0.2	0.0	3.18	<0.001
	ERA group	3	0.2	0.1	8.67	<0.001
	Residuals	935	6.3	0.0		

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Pair 1	Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
	YoB	11	541406.0	49218.7	21.86	<0.001
	Allelic group	3	35962.0	11987.2	5.32	0.001
	Residuals	934	2102803.0	2251.4		
	YoB	11	541406.0	49218.7	21.87	<0.001
	ERA group	3	37097.0	12365.5	5.50	0.001
	Residuals	934	2101668.0	2250.2		

Pair 3	Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
	YoB	12	407778.9	33981.6	11.64	<0.001
	ERA group	3	35271.9	11757.3	4.03	0.008
	Residuals	289	843588.6	2919.0		

Lean conformation EBV

Pair 3	Term	Df	Sum of Sq	Mean Sq	F value	Pr(F)
	YoB	12	57.2	4.8	14.28	<0.001
	ERA group	3	3.9	1.3	3.90	0.009
	Residuals	289	96.4	0.3		

Appendix 5 The number and percentages sired by each ram

Rams highlighted in bold indicated shared rams in Sire Reference Schemes

Farm D34		
Ram	Number	%
K31	1	0.2
K52	2	0.4
S51	41	7.9
T76	14	2.7
L12	15	2.9
L40	1	0.2
U45	3	0.6
X116	18	3.5
N138	3	0.6
J30	1	0.2
T91	**1**	0.2
W163	**12**	2.3
N255	4	0.8
S343	2	0.4
T122	**6**	1.2
U241	**4**	0.8
L2	3	0.6
T10	1	0.2
U28	1	0.2
U36	2	**0.4**
L14	2	0.4
L44	2	0.4
N163	6	1.2
N167	**5**	1
P158	15	2.9
P163	3	0.6
S119	5	1
S16	**5**	1
S168	**34**	6.6
S379	14	2.7
S47	4	0.8
T205	3	0.6
U107	4	0.8
U112	27	5.2
U177	5	1
U213	26	5
U6	**23**	4.4

Farm D34 cont.		
Ram	Number	%
W19	6	1.2
W231	22	4.2
W48	**23**	4.4
X318	13	2.5
L8	17	3.3
P13	16	3.1
T7	25	4.8
P13	**14**	2.7
6589	**11**	2.1
K8	31	6
361	**3**	0.6
478	**13**	2.5

Farm H19		
Ram	Number	%
P108	20	4.6
H17	3	0.7
N22	3	0.7
P46	19	4.4
S26	4	0.9
S8	1	0.2
T141	22	5.1
T91	**23**	5.3
U112	9	2.1
U141	5	1.2
U21	10	2.3
U65	17	3.9
W124	2	0.5
W163	**8**	1.9
W183	4	0.9
K65	16	3.7
L130	22	5.1
S343	12	2.8
T122	**8**	1.9
U241	**5**	1.2
N22	26	6
N167	**6**	1.4

Farm H19 cont		
Ram	Number	%
S16	**12**	2.8
S168	**6**	1.4
S74	12	2.8
U6	**13**	3
W48	**1**	0.2
P13	**18**	4.2
6589	**4**	0.9
K17	32	7.4
N23	21	4.9
361	**8**	1.9
478	**38**	8.8

Farm B11 Texel		
Ram	Number	%
0	2	0.8
1	30	12.3
7015	7	2.9
7023	32	13.1
8020	10	4.1
9001	5	2
9023	15	6.1
91	51	20.9
BA	9	3.7
Bar.Ad	7	2.9
CC	6	2.5
CE	19	7.8
CG	1	0.4
HC	20	8.2
LA	9	3.7
LC	6	2.5
MVB	8	3.3
PT	7	2.9

Farm T49		
Ram	Number	%
5042	1	1.0
6040	**1**	1.0
8148	**3**	**3.0**
8048	1	1.0
7099	1	1.0
8011	12	12.1
3018	1	1.0
1068	**2**	2.0
3041	18	18.2
4149	3	3.0
6032	1	1.0
6041	**2**	2.0
9066	**2**	2.0
7044	8	8.1
5046	4	4.0
6013	4	4.0
7002	1	1.0
7012	19	19.2
8127	7	7.1
9072	4	4.0
4015	3	3.0

Farm S03		
Ram	Number	%
6040	**1**	0.5
7041	19	9.2
8148	**4**	1.9
9311	5	2.4
4032	1	0.5
8001	1	0.5
5067	1	0.5
5015	6	2.9
6007	1	0.5
9023	9	4.4
5137	1	0.5
5006	22	10.7
9041	2	1.0
1068	**2**	1.0
7117	1	0.5
5003	1	0.5
4202	1	0.5
7029	1	0.5
2030	1	0.5
1075	2	1.0
90	5	2.4
3010	1	0.5
7002	1	0.5
7109	2	1.0
9013	5	2.4
6080	5	2.4
6041	**19**	9.2
1094	1	0.5
9066	**6**	2.9
4058	16	7.8
7012	2	1.0
2001	5	2.4
4036	1	0.5
7012	6	2.9
8004	1	0.5
9039	1	0.5
25	1	0.5
6057	3	1.5
8064	1	0.5
5003	1	0.5
8046	2	1.0
8004	34	16.5
8017	1	0.5

Farm P27		
Ram	Number	%
91235	1	0.5
93034	1	0.5
95062	2	1.0
95080	1	0.5
96047	3	1.4
95033	13	6.3
98014	3	1.4
95075	2	1.0
98080	2	1.0
91027	1	0.5
87014	3	1.4
93039	1	0.5
95042	13	6.3
95070	4	1.9
97051	5	2.4
98063	15	7.2
95038	11	5.3
98015	2	1.0
94026	17	8.2
97057	18	8.7
99070	16	7.7
94076	3	1.4
19	4	1.9
46	10	4.8
50	12	5.8
97	1	0.5
95050	2	1.0
97060	1	0.5
98081	4	1.9
98127	9	4.3
99009	4	1.9
99042	1	0.5
99088	2	1.0
92118	3	1.4
93033	1	0.5
96033	2	1.0
95013	1	0.5
98059	14	6.7

Appendix 6 Gamma Distributions

6.1 The values of the gamma distribution used in generation of the variation of the values of R_0 for the Charollais and Texel flocks.

2.975	0.634	0.109	0.676	1.130	0.138	0.356	1.633	1.554	0.197
0.676	0.236	1.079	0.110	0.551	1.132	0.772	0.348	0.185	0.263
0.731	1.357	1.441	0.127	0.054	0.977	1.398	0.250	0.891	3.078
0.179	0.044	0.011	1.415	1.039	2.005	0.253	0.918	1.807	1.135
0.435	0.834	3.567	0.229	0.005	0.466	0.026	0.912	0.139	4.332
0.089	1.244	1.017	0.088	0.133	1.393	1.125	2.798	1.391	1.809
0.560	2.980	0.294	1.442	0.293	0.655	0.692	0.632	2.820	0.309
0.011	0.758	0.643	1.115	0.824	1.381	1.718	0.466	1.196	1.189
0.018	0.226	1.755	0.508	1.427	5.843	0.330	0.415	0.647	1.860
0.289	1.183	0.487	1.013	1.419	0.767	1.923	0.442	0.629	0.073
0.272	0.780	0.349	0.658	0.127	1.037	0.085	0.682	3.199	6.132
1.472	0.526	0.825	0.794	0.046	0.035	0.497	0.343	0.280	0.435
0.287	0.328	0.275	0.429	1.048	1.312	0.570	2.506	2.029	1.725
0.307	1.918	4.155	0.198	0.384	0.337	2.025	0.044	0.534	1.629
0.218	1.087	0.040	0.222	0.044	1.062	4.964	0.191	4.018	1.068
0.873	0.983	0.022	0.385	0.123	0.051	0.135	1.780	0.078	0.119
0.501	0.333	3.449	1.628	0.098	0.011	2.063	0.399	0.867	0.116
0.440	1.259	0.333	0.191	0.578	0.369	0.731	0.853	0.466	0.402
0.200	0.614	2.054	0.839	1.238	0.275	0.664	0.187	1.289	0.425
0.081	0.342	0.196	0.018	1.405	0.263	0.100	0.627	0.551	0.512
1.366	1.645	1.726	1.119	0.403	1.427	0.375	0.201	1.259	1.134
1.281	0.212	2.166	1.542	0.601	0.121	1.874	0.390	0.529	0.185
0.337	0.625	0.310	0.165	1.724	1.005	2.515	0.988	0.285	0.936
2.778	0.011	3.930	0.601	1.074	1.309	0.387	0.151	0.843	0.958
1.195	1.412	3.892	1.020	0.591	0.591	0.054	0.319	0.277	0.351
2.548	2.409	0.768	2.280	0.226	1.688	1.432	2.230	0.246	1.127
2.869	0.541	0.490	4.112	0.779	0.189	0.259	0.268	0.947	1.894
2.743	0.449	0.061	1.148	0.204	0.238	0.302	0.472	0.381	0.457
0.296	1.849	0.622	3.406	1.077	0.462	1.792	0.032	0.305	3.248
1.685	0.271	1.476	0.101	0.297	0.494	1.902	0.612	0.627	0.073
0.029	1.246	0.251	0.565	0.158	1.611	0.615	0.071	0.008	1.281
0.183	0.876	0.113	1.152	0.669	0.901	0.295	0.043	1.833	0.992
2.112	0.748	0.324	0.935	1.304	0.400	1.521	1.306	0.054	0.193
1.070	0.340	0.363	0.020	3.126	0.332	0.303	0.809	1.891	0.711
0.992	0.435	0.343	0.962	3.343	0.639	0.502	0.930	1.703	0.175
0.252	0.251	0.304	3.449	0.689	2.102	1.198	0.113	1.416	0.211
0.829	0.204	1.313	1.567	0.563	0.270	0.755	4.216	0.955	0.994
1.373	1.124	0.622	3.208	0.419	0.055	0.224	0.827	0.035	0.202
1.633	0.196	5.021	0.912	0.131	0.721	1.030	1.366	0.617	0.326
0.338	0.154	2.507	1.464	1.101	0.777	0.534	0.803	1.009	2.541
0.846	3.014	0.067	0.046	0.189	0.632	2.647	0.179	0.138	0.334
4.015	0.460	1.361	0.356	0.882	0.479	0.253	0.558	0.477	1.222
0.556	0.487	0.036	0.664	1.797	0.310	0.622	0.228	1.067	1.140
4.048	5.261	2.010	2.492	2.107	1.251	0.334	0.483	1.295	2.767
0.872	0.105	0.568	0.463	1.853	0.602	0.604	2.469	3.112	0.121

1.284	0.297	3.845	1.385	1.841	0.153	4.165	0.056	1.270	2.050
0.539	1.453	1.913	1.058	0.613	0.644	0.915	0.530	3.710	2.275
0.261	0.347	1.270	0.571	0.025	0.635	0.197	0.145	1.599	0.772
4.005	3.136	0.154	0.690	2.119	1.895	0.159	0.887	0.084	1.370
0.315	0.927	1.848	0.283	0.332	0.033	0.498	0.286	0.255	0.302
0.104	1.482	0.860	0.290	0.376	0.060	0.503	0.656	0.782	4.001
1.142	1.423	1.003	0.413	1.658	2.641	0.265	0.352	0.535	0.811
0.411	2.286	0.004	0.732	0.097	0.266	1.369	0.465	0.784	0.376
1.075	0.442	0.939	2.052	0.774	1.104	1.462	2.276	1.353	0.394
0.348	0.019	4.144	1.236	2.219	1.019	0.710	0.301	0.233	0.056
1.337	0.739	1.275	0.869	0.222	0.175	0.075	0.193	1.271	1.323
0.610	1.676	0.283	0.749	0.248	1.290	0.784	0.198	0.008	1.209
0.077	1.544	0.122	1.056	0.835	1.189	1.222	0.251	0.079	0.701
0.744	2.172	0.388	0.219	0.138	0.083	0.455	0.517	0.237	0.412
0.118	0.144	0.144	0.030	0.525	0.044	0.323	0.479	0.007	1.302
0.562	0.623	0.743	0.570	0.506	0.935	0.440	0.613	0.254	0.254
0.688	0.548	0.011	0.091	0.990	1.020	0.166	3.036	0.167	1.615
0.919	3.774	0.457	0.831	1.533	0.394	0.949	0.011	0.102	0.433
0.447	0.455	0.553	0.175	0.440	0.058	1.469	1.215	1.315	2.449
1.273	1.122	0.171	0.142	0.386	0.902	0.289	0.401	0.180	1.357
2.207	0.789	3.335	0.165	0.059	2.962	1.918	2.053	0.085	0.005
0.466	0.708	0.164	0.804	2.912	0.181	0.167	0.189	0.379	1.835
0.268	0.391	1.711	1.482	2.451	0.040	0.472	2.576	0.174	0.256
0.073	0.764	1.345	0.260	0.229	0.212	0.447	3.380	0.017	0.407
0.979	0.225	1.648	0.718	0.988	3.086	0.242	0.788	1.145	0.926
0.462	1.111	0.399	2.205	0.508	0.954	0.201	0.282	0.148	1.655
0.239	1.460	0.876	0.593	0.342	0.679	1.747	0.461	0.547	0.135
1.952	0.655	1.424	0.242	2.746	2.529	0.871	1.190	1.053	2.306
1.321	0.780	0.261	1.216	0.539	0.499	0.825	0.326	1.644	0.643
0.766	2.086	0.423	1.890	0.717	0.686	0.594	0.920	2.113	0.360
3.043	1.369	2.177	0.244	0.274	0.001	0.339	0.170	1.889	1.439
1.011	0.227	0.145	1.224	1.249	1.073	0.047	0.215	0.860	0.337
0.582	2.182	0.007	1.020	1.177	0.360	0.805	0.688	1.054	0.168
0.552	1.027	0.147	0.217	0.673	0.569	0.009	0.850	0.610	0.858
0.432	0.430	0.398	0.078	1.387	1.288	0.209	0.449	0.356	4.526
3.598	2.073	0.554	0.154	0.357	0.813	0.226	0.957	1.304	1.506
0.474	0.468	3.042	2.835	0.196	0.086	2.364	0.093	0.159	0.924
0.178	1.686	0.104	3.074	0.993	2.706	0.083	0.634	1.007	0.577
1.340	0.125	0.322	0.241	0.602	0.398	0.883	1.008	0.679	1.589
0.113	0.330	0.410	0.530	0.347	0.371	4.666	0.549	0.139	0.583
1.317	2.358	0.671	5.735	2.838	0.146	0.059	0.184	0.420	0.104
0.880	1.525	0.478	1.262	0.838	0.332	1.400	0.059	0.359	0.302
1.204	1.626	0.480	0.790	1.541	0.912	0.419	0.330	0.177	1.882
0.369	1.452	1.389	0.522	0.359	0.936	0.177	2.943	0.238	0.079
2.656	0.220	0.807	1.021	2.848	4.703	0.204	0.293	0.511	0.319
0.286	0.543	0.083	1.778	0.605	4.314	0.674	0.967	0.172	6.417
0.015	0.023	0.810	1.810	0.415	1.722	0.209	1.787	1.725	1.162
0.209	0.120	0.764	0.650	0.301	2.131	3.456	0.109	1.393	0.934
1.074	0.038	0.446	0.551	2.466	1.599	2.191	2.051	0.942	0.094
0.376	0.601	0.077	1.388	1.897	1.342	1.568	0.537	3.058	0.312
1.786	2.420	0.129	0.769	1.242	0.971	1.130	0.751	0.296	0.631
2.431	1.951	1.663	0.477	0.315	1.272	0.267	3.529	1.507	0.371

2.349	0.568	1.270	0.998	0.470	0.988	0.117	0.009	0.710	2.957
1.003	2.849	2.282	0.033	0.744	0.634	1.195	0.162	3.431	0.858
1.024	0.547	1.501	0.140	1.464	0.841	3.758	0.602	0.454	0.126
0.214	0.708	0.134	0.337	1.018	0.089	1.998	0.702	2.204	0.494
0.630	1.185	1.483	0.432	1.180	0.062	0.148	0.036	0.423	2.200
0.477	0.252	0.523	0.284	3.308	0.501	0.253	0.317	1.808	0.410
1.130	2.402	1.845	0.306	0.206	0.990	0.273	0.723	0.411	2.331
0.307	0.183	3.568	2.463	0.533	1.153	0.940	0.203	1.602	0.516
0.492	0.408	0.287	1.035	1.273	0.111	0.041	0.455	0.511	1.843
0.679	0.599	0.040	0.066	0.624	1.082	0.265	0.580	0.038	0.269
0.867	0.293	0.205	0.213	0.127	0.598	0.031	0.233	3.098	0.097
0.941	0.590	3.585	0.581	1.300	0.157	1.921	0.162	0.581	0.379
1.388	0.833	1.225	0.480	0.833	2.428	0.460	2.397	0.196	0.132
0.263	0.195	1.015	2.381	0.328	1.326	0.430	0.337	0.348	0.008
0.205	0.878	0.219	2.824	0.346	0.616	0.483	0.028	1.639	1.813
1.108	0.527	1.342	0.108	2.150	1.572	1.815	4.254	1.620	3.269
1.063	0.251	0.439	1.508	0.008	2.715	0.022	0.668	0.691	1.172
0.142	1.226	2.319	0.470	1.151	1.886	0.107	0.299	1.958	0.467
0.077	0.555	1.355	0.755	3.357	0.967	0.424	0.069	0.534	1.184
0.552	0.956	1.526	0.299	2.850	0.555	1.199	1.074	2.810	0.126
0.663	0.126	1.966	0.469	0.880	0.954	0.377	0.197	1.236	2.547
0.099	1.040	2.333	0.660	0.157	0.832	0.060	0.217	0.404	0.596
0.090	0.158	1.034	1.843	1.030	0.392	1.756	0.493	0.773	1.091
0.476	0.873	0.757	0.149	0.632	0.599	1.371	1.273	3.407	0.512
0.221	4.280	0.182	1.579	0.078	0.143	1.630	0.317	2.759	1.185
2.429	1.463	0.558	0.242	0.774	1.851	1.603	0.403	1.348	3.234
0.261	0.834	1.368	0.176	0.345	0.733	2.246	1.954	0.355	0.737
0.565	1.843	0.864	0.341	1.698	0.154	0.579	0.765	0.227	1.159
1.142	0.731	0.408	1.691	1.251	4.643	1.500	0.301	0.747	1.227
0.234	1.134	1.211	0.425	0.342	1.605	1.061	0.112	0.609	2.411
0.377	1.222	1.693	1.858	0.969	0.325	0.104	0.176	2.383	0.568
2.148	0.172	0.058	0.658	0.437	0.497	0.621	0.474	0.339	0.447
2.394	0.933	0.809	0.732	0.088	0.045	1.533	0.285	0.641	0.119
2.123	1.325	0.183	0.117	0.242	0.049	1.716	0.044	1.448	0.504
1.719	0.511	1.322	0.991	0.232	0.747	2.931	0.156	1.124	1.695
0.382	0.601	0.250	0.158	0.309	1.670	0.192	0.083	0.309	0.178
1.656	0.379	0.276	0.322	0.035	1.870	0.697	0.804	1.537	0.402
0.977	3.145	0.104	0.001	0.683	0.530	0.502	0.031	1.031	2.526
3.639	0.853	2.832	0.419	1.778	1.210	0.263	0.481	0.597	0.669
0.015	0.964	0.477	0.115	0.158	0.441	0.465	3.354	0.407	0.959
0.281	1.290	1.241	1.161	0.935	0.038	0.612	0.738	1.909	0.943
0.800	2.451	1.166	0.366	0.579	0.747	5.515	0.346	0.146	0.247
0.396	2.768	0.141	1.905	0.053	0.463	1.048	0.573	0.578	1.160
0.232	0.472	0.579	0.206	0.793	0.420	0.573	0.302	0.117	1.366
1.166	3.377	0.217	0.045	0.856	0.311	0.076	2.235	0.563	0.826
2.810	0.413	1.395	1.525	0.258	1.435	0.498	0.042	1.054	2.738
2.244	1.273	3.046	2.238	0.419	0.460	0.501	3.647	2.253	0.457
0.004	0.602	1.929	1.102	1.370	1.346	0.835	1.080	0.645	0.327
0.491	1.163	0.250	1.817	0.580	0.623	0.551	0.162	1.777	0.079
2.555	1.250	0.604	0.735	1.163	0.188	0.319	0.858	1.542	0.015
0.026	0.376	1.181	0.183	2.050	1.641	0.355	2.963	0.556	1.708
0.349	0.409	0.409	1.163	2.989	0.133	0.859	0.400	3.372	0.466

0.401	0.084	0.163	1.599	1.096	1.437	0.505	0.280	1.793	0.235

6.2 The values of the gamma distribution used in generation of the variation of the values of R_0 for the Swaledale flocks.

1.177	0.947	1.103	2.688	0.143	0.081	0.409	2.103	2.523	0.338
0.346	0.254	0.138	1.229	0.162	0.532	2.269	0.224	0.051	0.033
1.386	0.499	1.396	0.554	0.594	0.027	0.238	1.579	0.925	0.297
1.091	1.798	0.766	0.112	0.168	0.117	1.821	0.439	1.139	0.495
0.890	1.023	0.210	0.301	1.445	0.205	5.226	1.300	2.211	0.781
0.116	0.246	0.764	1.251	0.150	2.655	1.537	0.093	0.280	1.256
0.312	0.525	0.950	0.009	0.610	0.648	0.063	0.463	2.221	0.669
0.168	0.149	0.734	0.552	1.044	1.285	1.127	0.625	2.501	1.941
0.503	1.025	0.758	1.232	1.839	1.577	0.794	0.781	0.551	1.222
0.963	0.488	1.072	0.013	0.694	0.591	1.269	0.609	1.370	0.032
0.657	4.723	0.835	2.438	0.892	0.003	0.883	0.150	0.264	0.421
0.119	0.693	0.574	0.775	0.850	1.550	0.313	6.025	3.079	6.013
1.336	0.219	1.274	0.462	1.605	0.082	0.402	0.281	0.543	0.195
0.578	0.065	0.797	0.977	0.059	1.362	0.977	0.266	0.096	0.854
0.949	1.426	0.794	0.105	4.051	1.007	2.104	1.539	0.451	0.399
0.455	3.637	1.069	0.641	1.275	0.301	1.825	1.369	0.823	0.147
1.676	0.367	0.206	3.476	0.136	1.374	0.003	0.413	0.036	0.822
0.789	0.160	0.969	0.075	1.054	2.826	0.303	1.407	0.080	0.771
0.085	1.757	1.115	3.009	0.837	0.308	0.858	1.240	0.293	0.140
0.489	2.876	1.069	0.531	0.310	0.288	0.566	0.164	4.541	0.756
0.594	1.497	0.088	1.036	1.559	2.037	0.636	0.579	0.007	0.361
1.536	0.659	1.043	3.880	0.895	0.387	0.545	1.314	0.080	1.133
0.195	0.343	0.836	0.196	0.919	0.601	1.304	1.189	0.001	0.040
0.253	0.325	1.367	1.958	0.242	3.324	1.527	0.395	0.580	1.566
0.706	0.449	1.698	0.048	0.026	0.257	1.489	0.036	0.254	0.735
0.029	2.331	1.365	0.680	2.220	0.447	1.311	1.549	0.253	0.702
1.462	0.974	2.910	4.320	0.121	0.177	0.482	1.329	1.903	2.618
1.428	0.284	0.377	0.216	2.100	0.770	0.190	0.131	1.565	2.389
5.777	3.517	1.568	0.879	0.185	0.692	0.047	2.857	2.153	1.396
0.355	2.502	1.268	0.631	2.044	0.002	0.790	4.133	0.635	0.125
0.369	0.942	1.094	0.565	0.353	0.159	0.020	2.175	0.678	2.165
0.631	0.589	1.922	0.761	0.372	1.507	0.807	0.407	0.205	1.132
1.410	1.972	0.107	1.317	0.557	0.623	3.502	3.535	0.419	0.795
0.408	3.495	0.149	0.338	0.388	0.218	1.515	1.623	0.713	1.389
0.153	1.895	0.862	0.645	0.354	0.348	0.065	0.449	1.484	1.204
2.720	1.499	1.177	0.630	2.632	0.570	7.460	1.554	1.845	0.140
0.017	0.053	1.431	0.108	1.935	0.648	0.411	0.556	3.202	1.277
0.635	0.002	4.823	1.020	1.679	2.262	1.370	1.372	0.535	1.037
1.288	0.283	0.354	0.909	0.449	0.517	0.030	0.992	2.003	4.178
0.935	0.788	1.413	0.435	0.504	0.683	0.001	1.079	2.858	1.837
0.489	1.730	1.936	0.312	0.799	1.047	0.599	0.356	0.058	0.238
0.069	0.222	0.388	0.918	1.829	0.269	3.138	0.927	0.625	2.867
0.179	1.466	0.957	1.350	2.061	0.740	1.953	0.833	1.828	0.470
0.100	2.619	1.203	0.433	0.674	2.425	0.474	1.647	0.984	0.617
0.050	0.213	0.159	1.600	1.654	0.503	0.264	0.801	0.077	0.752

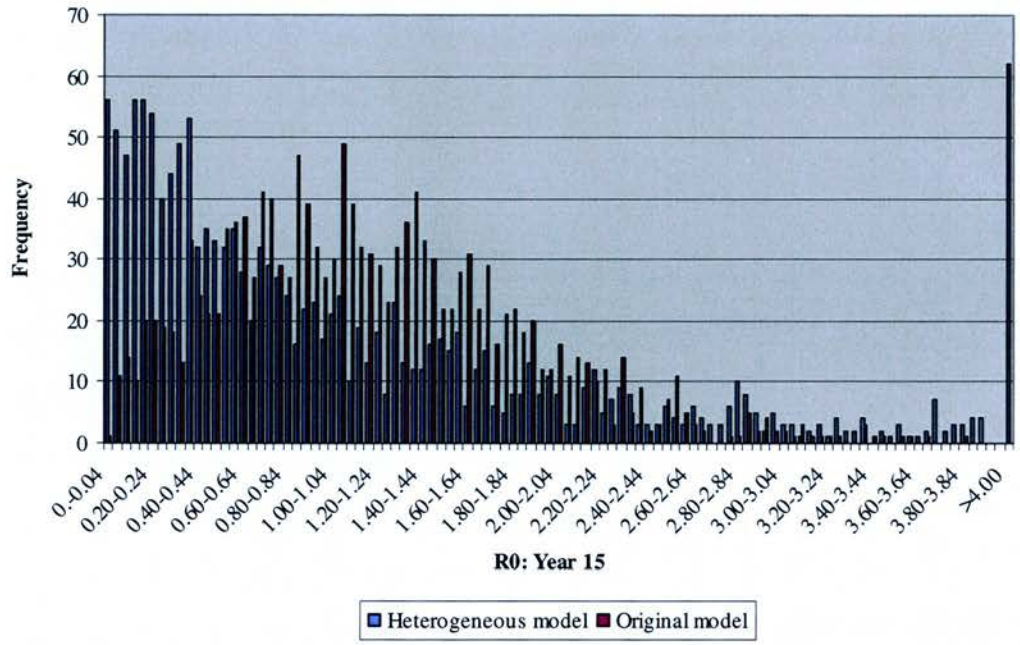
0.620	0.215	0.658	0.198	0.382	1.227	0.520	5.039	0.124	0.853
0.139	1.308	0.900	0.353	0.411	2.678	1.335	1.118	2.900	0.635
0.223	2.775	5.077	1.142	0.286	1.459	0.230	0.360	0.045	2.305
0.164	1.983	0.180	0.998	0.456	0.977	1.435	1.731	0.354	4.213
0.196	0.594	0.209	2.983	1.036	1.941	1.419	1.837	0.435	0.051
1.549	0.223	4.663	0.235	1.000	1.064	0.406	1.756	3.806	0.466
0.300	0.655	1.121	0.614	1.355	0.379	2.174	0.535	0.739	2.954
0.128	0.706	0.188	0.531	0.937	0.671	1.595	0.148	0.844	0.791
0.402	0.321	1.063	1.963	0.601	0.729	1.762	0.166	1.026	0.155
2.556	1.902	0.003	2.445	1.082	2.002	0.378	0.391	1.977	2.393
0.718	0.005	0.354	1.020	1.136	3.664	0.028	0.426	1.123	0.062
0.550	1.790	1.790	0.081	0.164	2.072	1.557	0.391	2.584	1.615
0.225	0.165	0.302	0.444	1.252	0.393	0.025	0.883	0.950	0.245
0.403	0.587	0.038	0.528	0.030	0.074	1.077	2.012	0.070	0.930
0.118	0.218	1.012	0.805	0.461	0.688	1.578	0.533	0.040	0.764
1.381	1.895	0.806	3.056	2.042	1.425	0.078	1.100	0.622	0.023
0.504	0.792	0.540	0.500	0.130	0.442	2.399	0.626	0.054	0.483
2.569	0.375	0.083	0.942	1.163	0.009	0.017	1.919	0.023	1.539
0.257	1.847	0.064	0.428	0.190	0.675	0.182	0.936	1.751	0.773
0.452	1.718	0.051	0.342	0.135	2.998	0.236	0.769	2.333	0.287
0.297	0.829	0.078	2.934	1.183	2.855	0.216	1.629	0.005	1.186
0.112	0.073	0.483	3.984	1.192	0.246	0.116	1.631	1.476	0.042
0.161	1.903	0.249	0.727	1.165	0.038	2.454	0.034	1.860	0.774
0.247	1.046	0.074	2.834	0.879	1.586	0.165	0.142	0.546	0.233
0.297	0.661	0.181	1.128	0.260	0.666	0.917	1.339	1.183	0.056
1.574	0.006	0.646	3.182	0.460	0.322	0.481	0.346	0.176	0.504
0.126	0.578	2.394	0.963	0.904	0.412	0.091	1.382	0.805	0.643
0.122	0.155	0.087	0.833	0.231	0.282	0.527	1.295	0.296	0.774
1.216	1.078	0.298	0.537	1.536	3.218	0.222	1.215	0.228	0.463
0.235	2.546	0.195	0.493	1.012	1.415	0.541	2.297	0.571	0.360
1.520	0.431	0.533	0.058	0.532	3.303	1.043	0.403	0.770	1.569
0.476	0.023	1.618	1.195	0.043	0.175	2.006	0.306	0.681	0.617
2.152	0.122	0.730	0.480	1.173	0.403	0.212	0.283	0.800	0.693
0.651	5.437	1.443	1.254	0.269	0.798	0.486	0.535	0.914	0.083
0.673	0.309	1.232	5.458	0.678	0.089	0.232	1.086	0.622	0.081
0.993	4.726	0.289	1.963	1.012	1.235	0.700	2.259	0.454	0.051
0.039	1.489	1.378	1.308	0.216	0.054	1.638	1.412	0.996	1.940
0.426	0.378	0.729	0.573	0.102	0.400	2.432	2.130	1.341	0.799
3.435	0.080	0.098	3.409	0.841	1.938	0.467	1.224	0.000	5.107
0.268	0.058	1.235	0.128	0.381	0.533	0.474	0.444	0.969	1.665
0.533	0.185	0.103	4.394	1.607	0.919	2.306	0.615	0.192	0.572
0.016	0.087	0.526	0.322	1.030	3.237	0.514	0.233	0.547	2.386
2.066	0.211	0.350	0.013	0.234	0.807	0.096	0.248	0.569	0.283
2.268	0.321	0.967	0.205	0.173	0.888	0.232	1.277	0.336	1.507
0.151	0.162	1.092	1.943	1.171	2.347	0.297	0.772	1.102	0.297
0.212	0.328	1.641	2.603	0.244	0.904	1.943	0.738	0.215	0.839
1.683	3.515	0.730	0.033	0.467	1.576	0.147	0.932	1.056	1.835
0.152	0.393	0.723	1.936	3.621	0.030	0.184	0.268	1.392	0.875
1.216	0.149	1.989	2.315	1.249	0.025	1.318	0.508	0.811	0.532
0.738	0.350	1.106	1.147	1.690	0.125	0.792	2.042	0.261	0.155
1.005	0.878	0.600	1.138	0.687	0.706	0.281	0.141	0.334	0.929
0.006	1.030	1.875	0.953	0.050	0.096	0.042	0.321	0.597	0.592

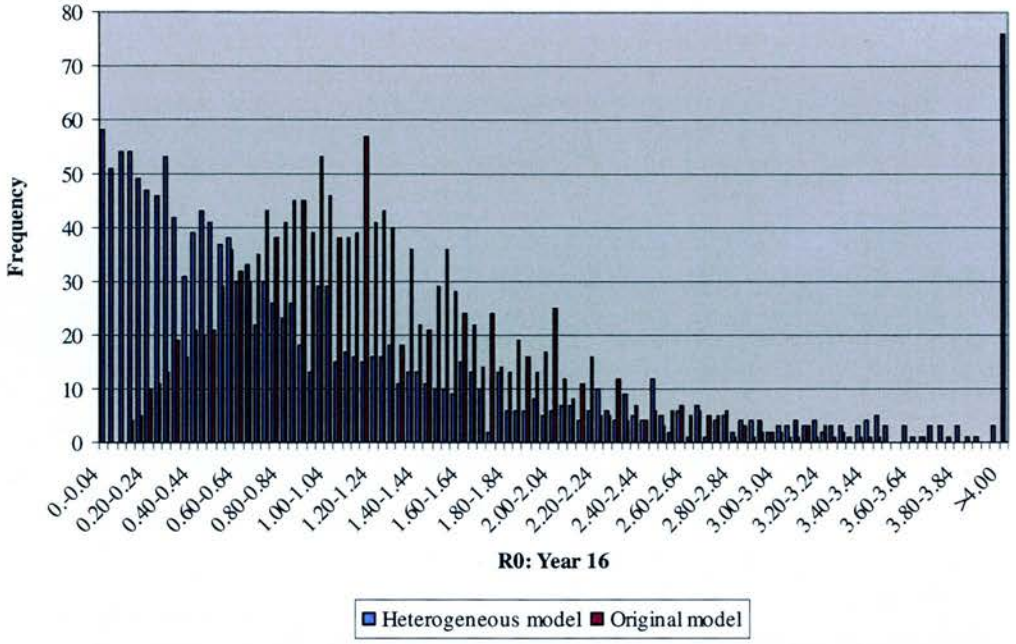
0.196	0.998	1.066	0.324	1.821	0.698	0.396	0.847	1.321	1.778
2.406	0.597	3.392	2.140	1.161	0.506	0.417	1.320	1.153	1.559
3.385	2.373	0.725	0.145	0.254	0.362	0.755	1.315	0.169	0.248
0.495	0.239	0.733	0.760	0.150	2.337	2.595	0.186	0.886	2.189
0.381	0.357	0.066	1.052	0.612	2.309	0.224	1.078	1.514	1.863
0.609	2.131	0.790	0.017	2.451	0.881	2.871	1.487	0.151	0.918
0.592	1.111	1.922	1.596	0.721	0.318	0.600	0.989	1.207	0.514
0.831	0.049	0.258	0.028	0.265	0.251	3.811	1.344	0.814	1.047
0.682	0.270	0.324	1.467	0.583	1.772	0.610	0.821	1.843	0.180
2.093	0.452	1.415	0.242	0.318	0.621	0.582	2.161	0.129	0.004
0.588	0.019	1.054	0.179	1.364	0.228	0.275	0.146	1.077	2.450
1.228	1.691	1.826	0.767	1.297	1.724	1.363	2.434	0.681	4.415
0.289	0.491	0.554	0.034	1.946	0.013	1.114	0.062	1.007	0.612
0.041	0.511	0.089	0.708	0.457	0.767	1.708	0.438	0.635	1.106
1.987	1.456	1.324	0.128	1.301	0.483	0.315	1.218	0.568	0.099
0.706	1.743	0.331	1.123	0.061	0.864	1.755	1.192	0.581	1.966
2.674	0.122	0.967	0.108	1.756	0.156	0.444	0.220	1.567	0.818
1.155	0.175	1.129	2.322	2.631	3.003	0.922	0.920	0.409	1.401
4.262	0.171	0.075	0.359	2.502	0.222	0.674	3.427	1.559	0.012
1.648	0.847	1.449	3.382	0.233	0.938	0.386	2.342	0.683	2.385
1.526	0.566	1.261	2.618	0.051	0.216	0.650	1.203	0.332	0.256
1.329	0.285	0.699	1.499	0.887	0.920	0.317	0.304	2.034	0.841
0.234	2.744	0.068	3.244	0.500	0.389	0.018	1.273	0.423	0.297
1.437	0.999	0.110	0.138	0.364	0.057	0.209	0.073	1.504	0.848
0.768	4.391	0.475	1.661	0.347	0.616	1.064	6.497	0.845	1.102
0.212	0.845	1.521	0.594	0.036	0.248	0.022	1.657	1.298	0.020
1.544	0.536	1.651	0.826	0.082	0.428	0.024	2.324	4.207	0.113
0.085	1.050	0.472	0.641	0.473	0.048	0.735	0.827	2.036	0.288
0.615	0.370	0.236	1.525	0.904	1.333	0.347	2.129	0.797	0.018
2.416	0.317	0.378	0.256	0.377	0.314	1.724	0.408	1.267	1.276
0.053	2.990	2.392	1.789	0.474	0.023	1.363	0.807	1.177	2.119
2.318	0.227	1.400	0.669	0.075	1.013	1.376	0.319	0.186	1.174
3.215	0.072	1.495	1.317	2.596	0.772	2.553	1.044	0.807	0.356
0.651	1.116	0.691	1.518	0.325	0.017	2.069	0.793	0.167	0.822
1.050	0.467	3.790	1.044	0.515	0.070	1.354	0.622	0.092	0.217
1.023	1.621	0.110	0.681	0.414	0.615	0.650	2.545	0.339	0.328
0.420	0.096	1.063	1.635	2.563	5.704	0.788	3.824	1.915	0.359
0.214	0.005	1.152	1.199	0.762	2.446	0.733	1.973	1.126	5.731
4.203	5.020	0.754	0.198	1.334	0.717	0.141	0.429	1.178	1.081
0.408	0.103	0.064	0.559	2.058	2.309	2.575	2.633	0.760	0.458
1.062	0.787	0.127	1.859	0.887	3.187	0.234	0.445	0.521	0.068
0.080	0.006	1.441	0.524	0.364	1.046	1.670	0.006	0.496	1.626
0.412	2.621	1.473	0.646	0.304	0.874	2.395	0.562	0.097	0.424
0.748	2.007	1.205	0.104	0.865	0.451	1.085	0.117	1.309	0.666
0.185	0.373	2.111	0.483	0.388	0.273	1.515	1.295	0.145	0.541
1.756	0.920	0.680	0.940	1.760	0.776	1.291	0.864	0.121	0.463
1.475	0.635	3.188	0.363	1.198	1.394	0.398	2.126	0.001	1.482
0.687	0.502	0.879	2.025	0.656	0.657	1.078	0.359	0.219	0.074
0.797	0.508	3.285	0.141	1.262	1.204	0.059	0.197	1.452	0.768
5.146	1.122	0.862	0.680	4.437	0.266	0.440	0.574	0.745	0.869
2.684	2.269	0.552	0.201	1.554	1.693	1.048	0.108	0.684	2.012
0.167	1.037	0.144	5.760	1.957	0.495	0.374	1.693	1.018	5.102

1.031	0.358	2.006	0.590	0.871	1.071	0.975	0.939	0.484	0.716
3.352	1.649	1.720	0.110	0.674	0.193	1.367	1.876	0.453	2.047
1.628	1.955	0.132	0.467	0.010	0.259	0.043	0.516	2.202	1.658
3.892	1.750	0.953	0.646	3.074	0.647	1.890	1.162	0.240	0.266
0.240	1.953	0.127	0.068	0.674	0.766	0.808	0.336	0.018	0.005
0.690	0.036	0.254	0.472	0.699	0.641	0.638	1.367	0.746	0.111
0.290	0.247	0.313	1.375	1.537	1.123	1.129	1.754	3.259	0.342
0.443	0.922	1.671	0.590	0.035	0.047	2.404	0.353	2.914	1.246
0.854	2.099	0.152	0.977	0.622	0.456	2.316	0.151	0.141	0.656
0.754	0.042	0.270	0.083	0.434	1.207	1.481	1.361	1.004	0.867
0.158	0.491	0.293	0.860	2.673	1.111	0.291	1.129	0.321	0.858
0.067	0.668	1.375	1.260	1.406	0.775	0.143	0.049	0.157	0.363
0.338	3.408	5.029	2.532	1.087	0.059	0.665	0.899	0.456	0.144
0.122	0.434	0.662	5.004	0.546	0.046	0.066	0.358	0.349	0.243
0.850	2.670	2.163	0.411	0.834	1.963	2.453	1.353	0.213	2.059
0.206	0.422	0.806	1.319	1.017	0.623	3.794	1.791	0.005	0.493
1.814	0.307	0.341	1.552	1.655	0.048	1.815	0.332	0.062	1.050
0.492	0.652	0.692	0.547	1.725	0.217	0.454	0.145	0.529	0.167
3.958	1.770	0.406	0.940	3.619	0.257	0.413	0.656	2.098	1.119
0.102	0.695	0.460	0.773	2.132	0.148	2.370	0.539	1.722	0.140
0.253	1.570	1.606	0.326	0.912	0.857	0.640	0.040	0.641	0.605

Appendix 7 The comparison of original and heterogeneous values of R_0 for the three sheep breeds under consideration between years 15 – 35.

7.1 Charollais




Frequency
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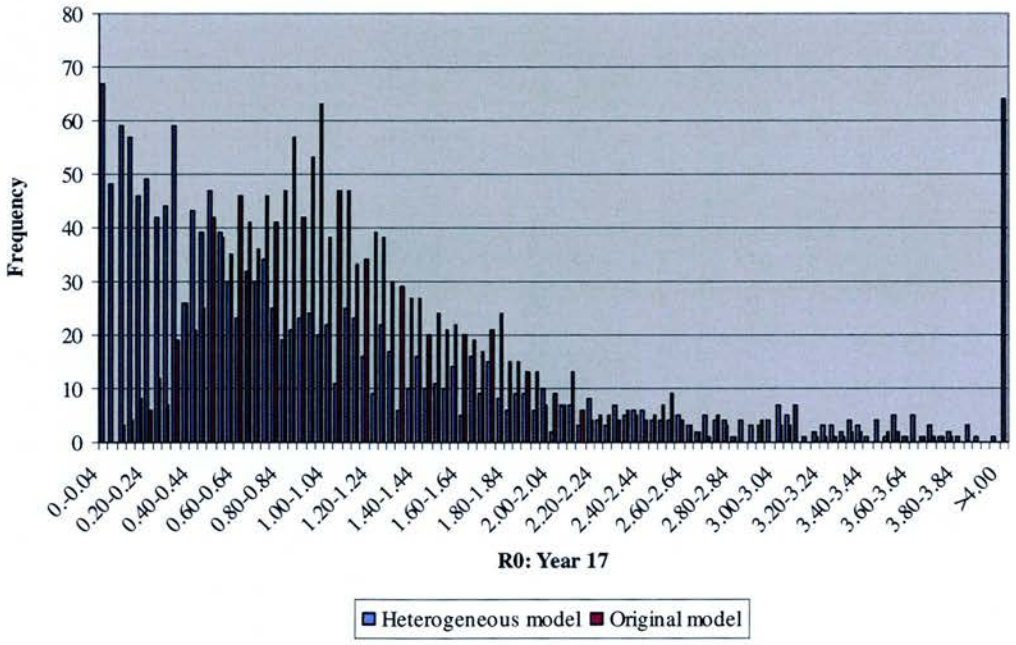
70
60
50
40
30
20
10
0
0,-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 16
Heterogeneous model
Original model


Frequency
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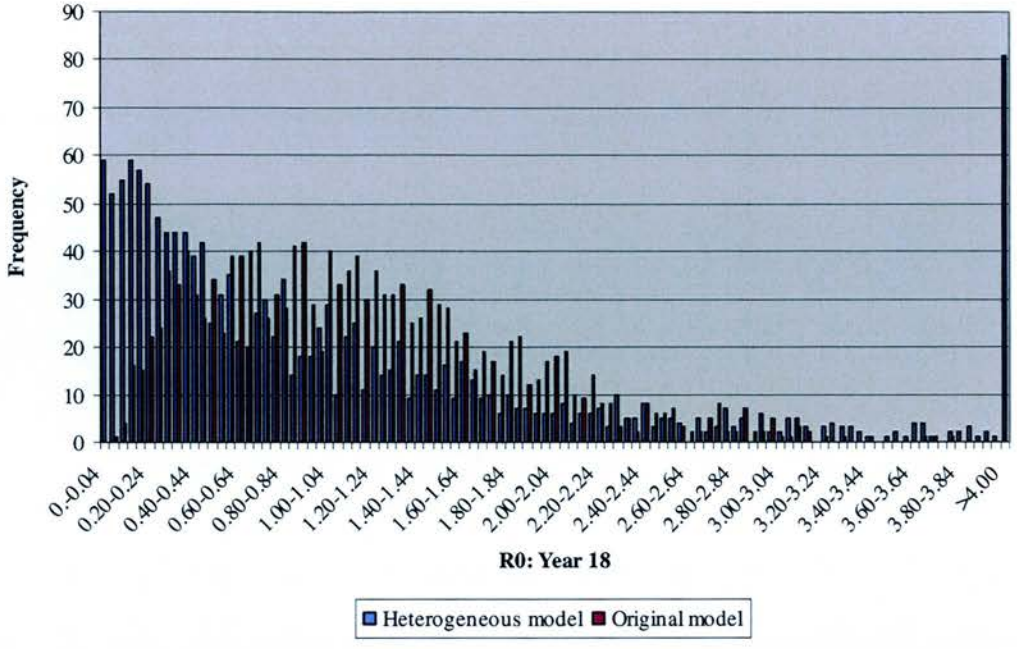
70
60
50
40
30
20
10
0
0,-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 17
Heterogeneous model
Original model


Frequency
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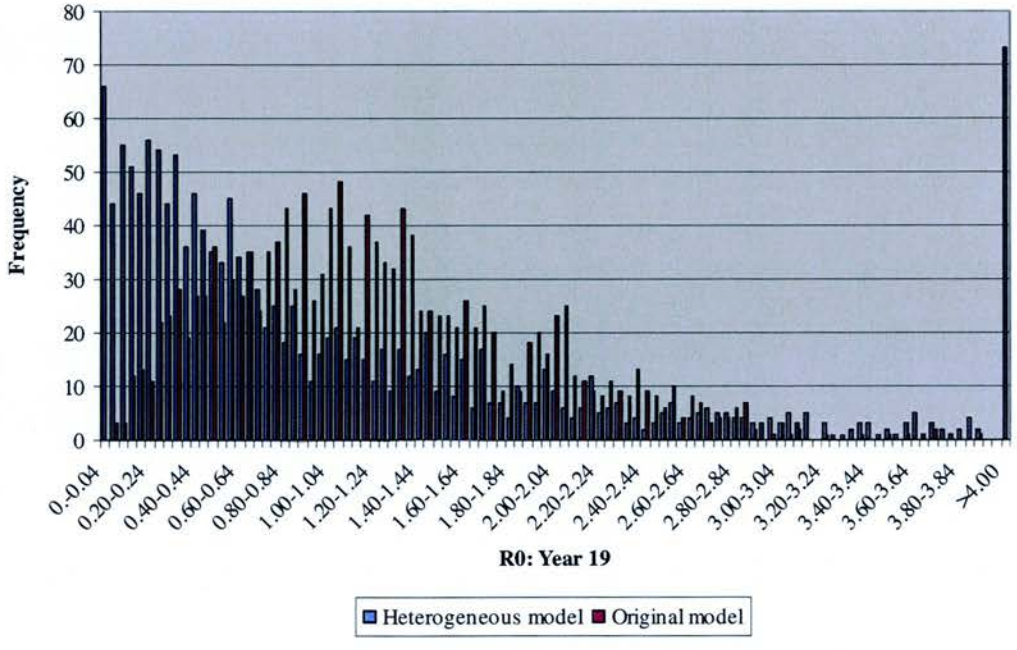
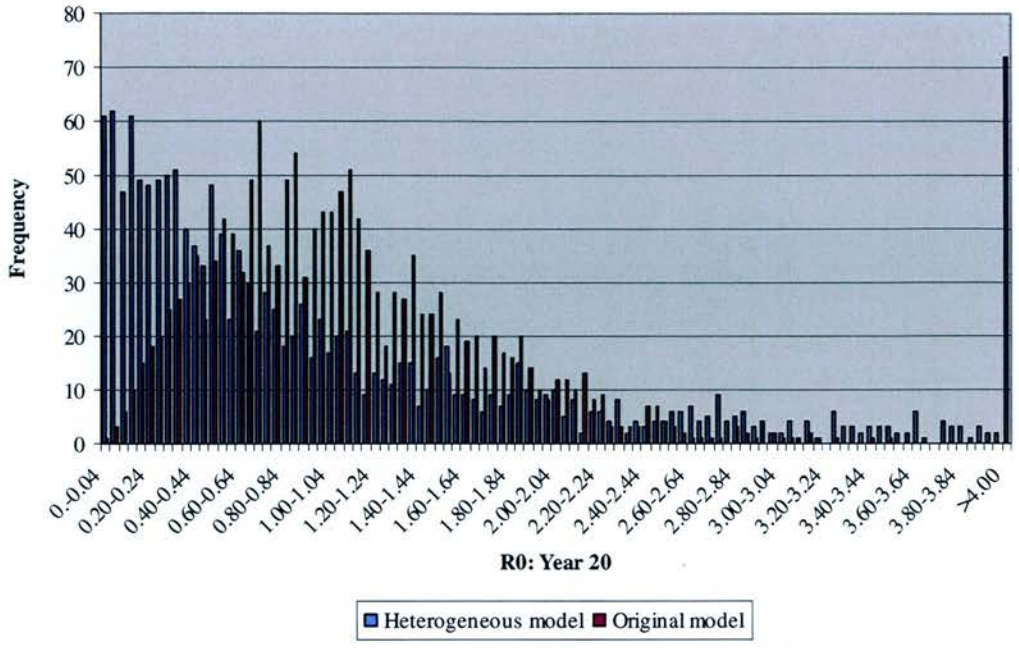
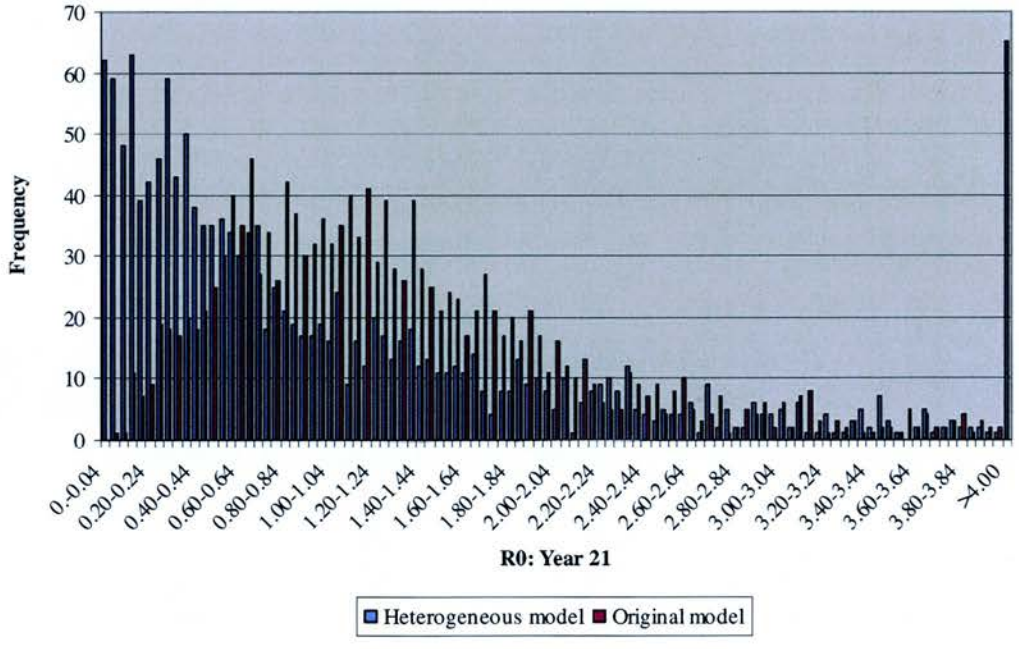
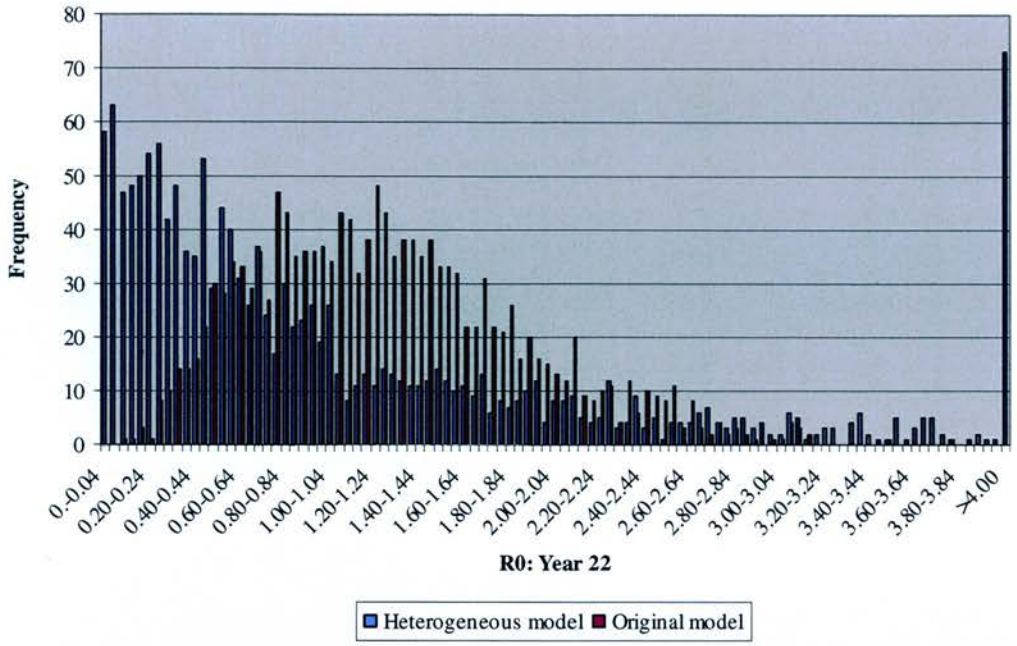
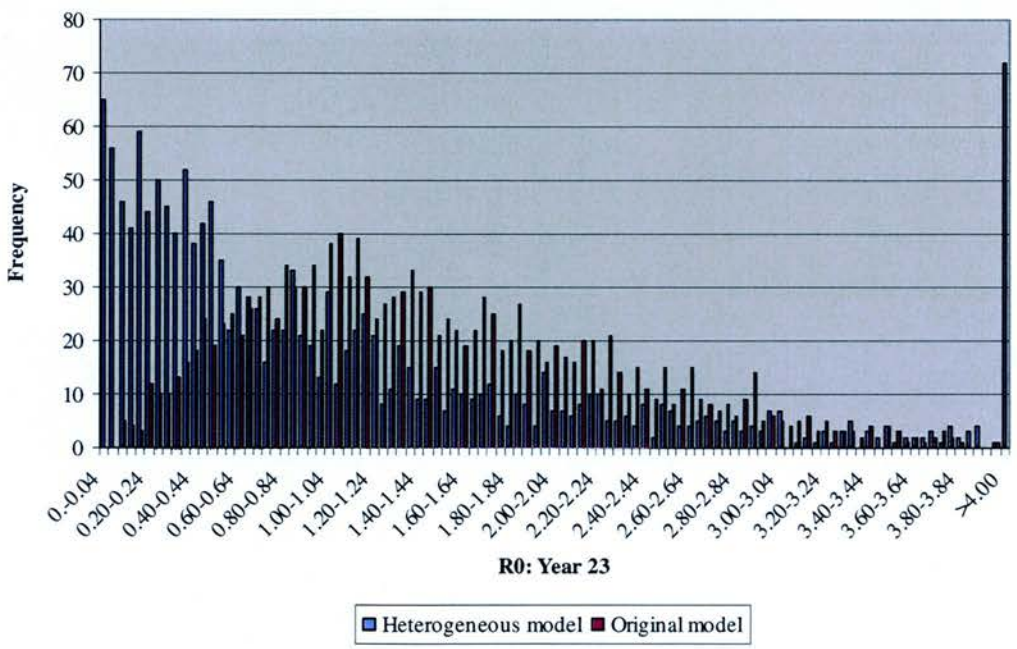
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 18
Heterogeneous model
Original model

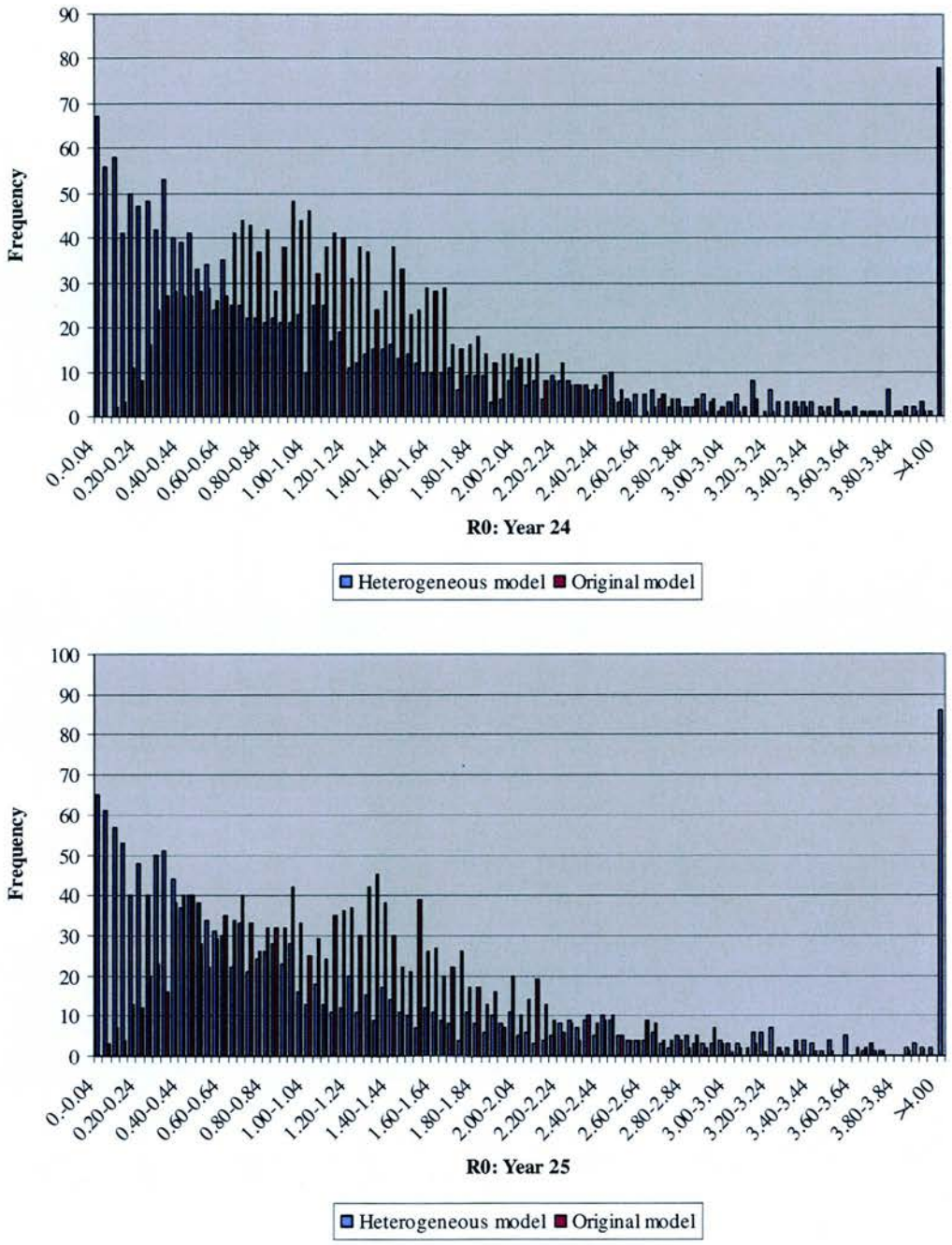

Frequency
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 19
Heterogeneous model
Original model


Frequency
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 20
Heterogeneous model
Original model

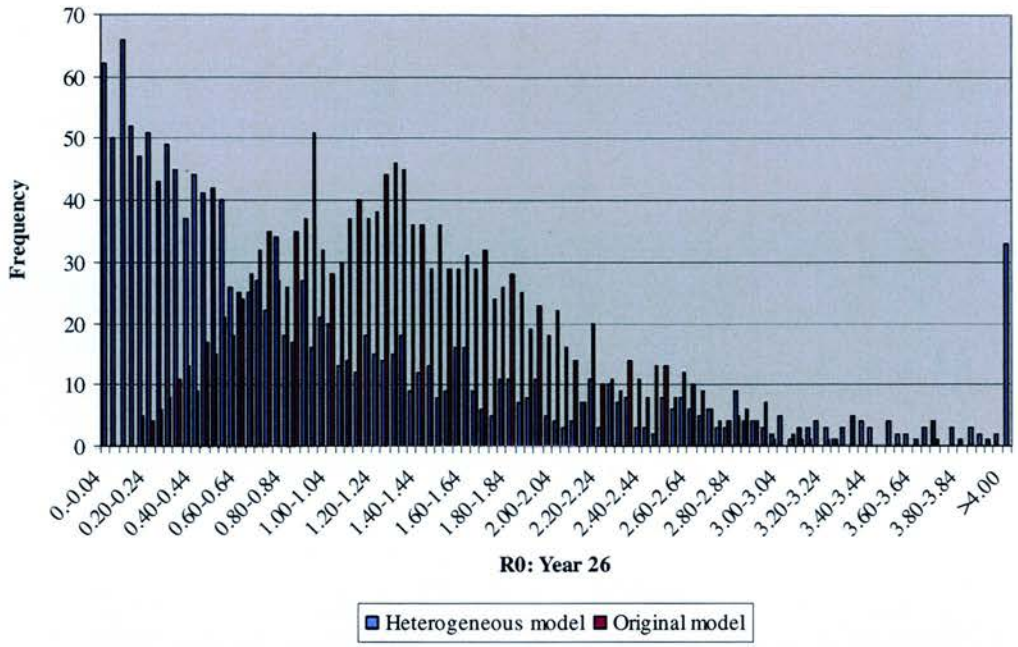

Frequency
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 21
Heterogeneous model
Original model

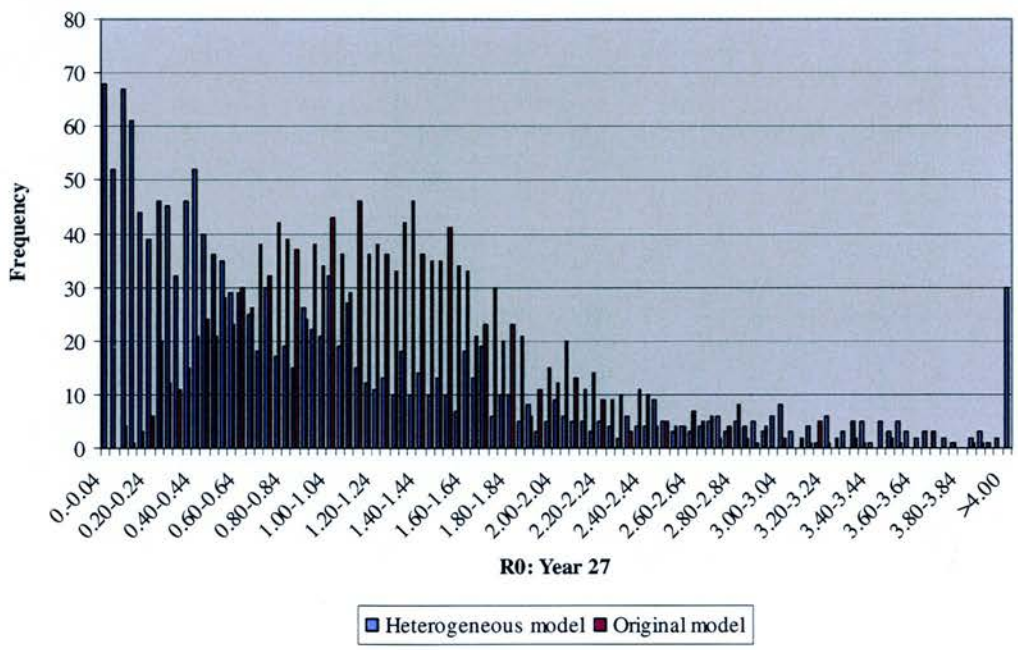

Frequency
80
70
60
50
40
30
20
10
0
0-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 22
Heterogeneous model
Original model

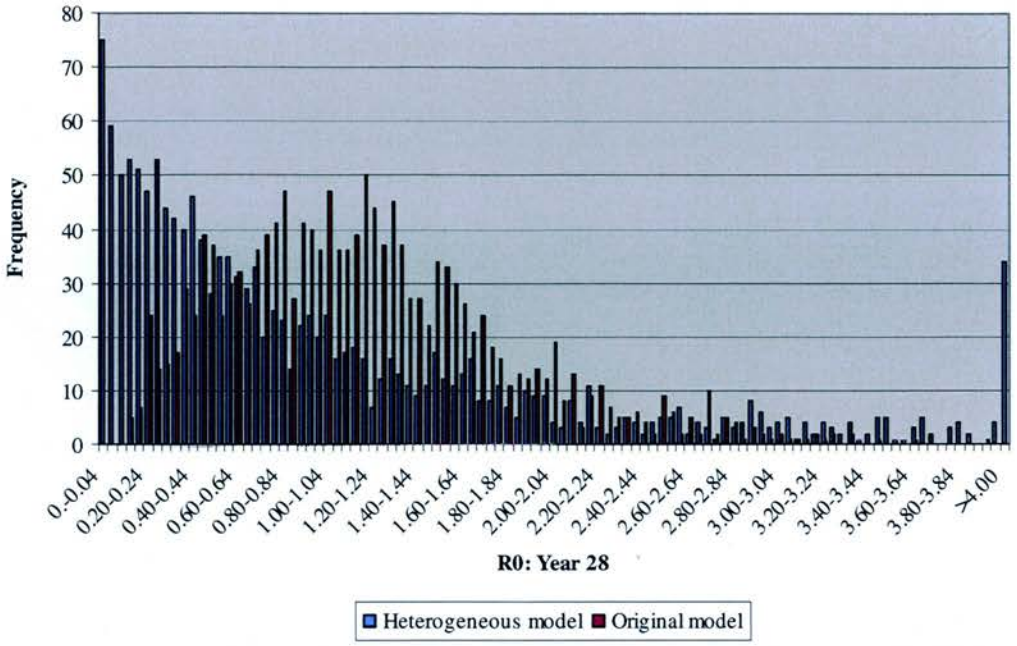

Frequency
80
70
60
50
40
30
20
10
0
0-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 23
Heterogeneous model
Original model

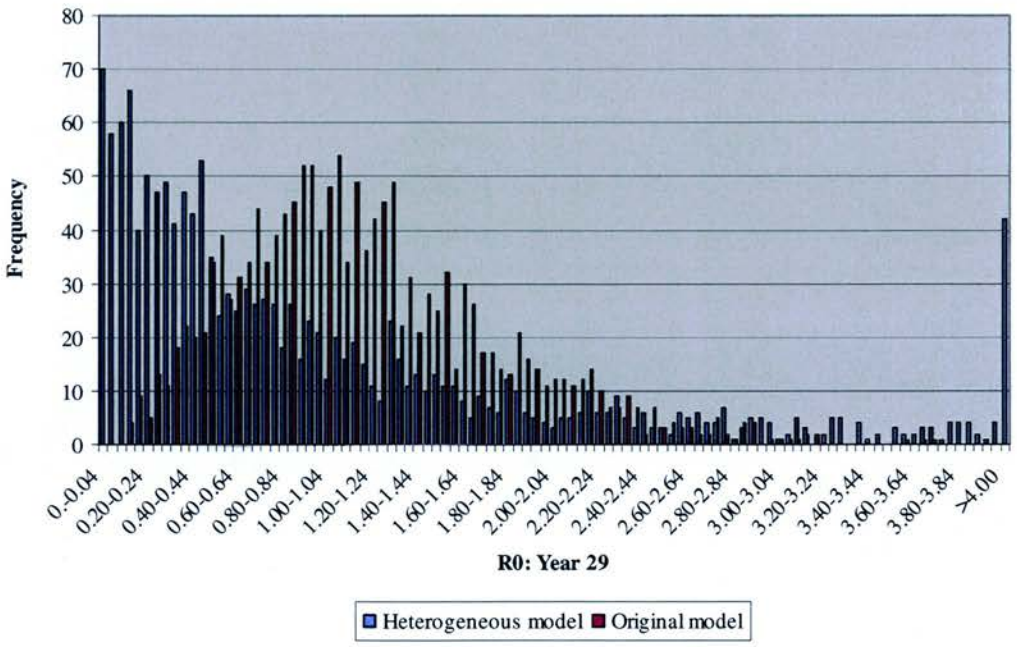

Frequency
90
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 24
Heterogeneous model
Original model

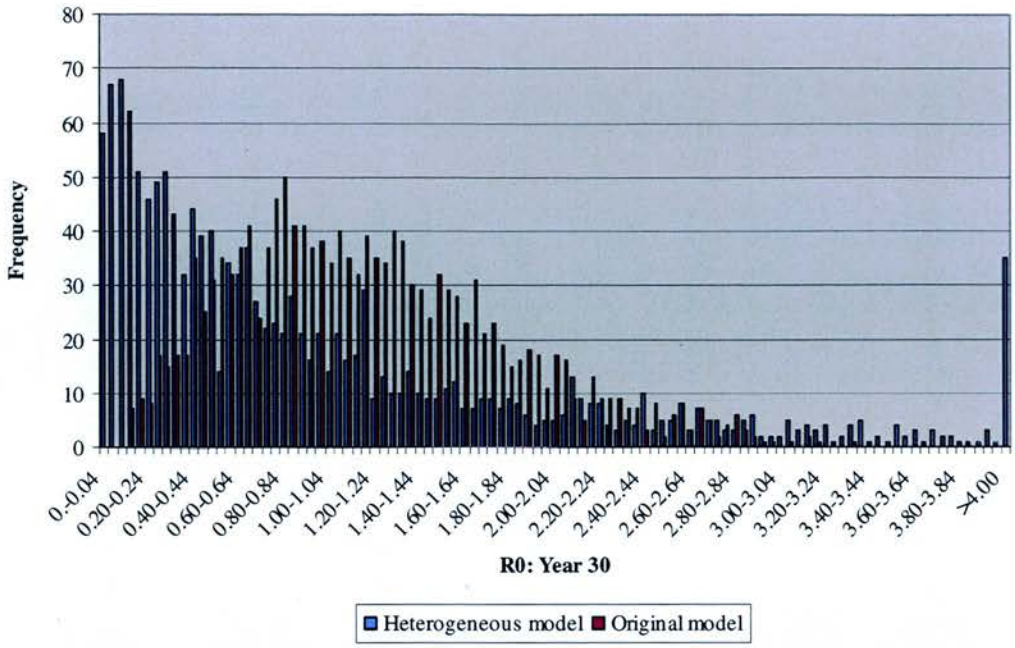
Frequency
100
90
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 25
Heterogeneous model
Original model

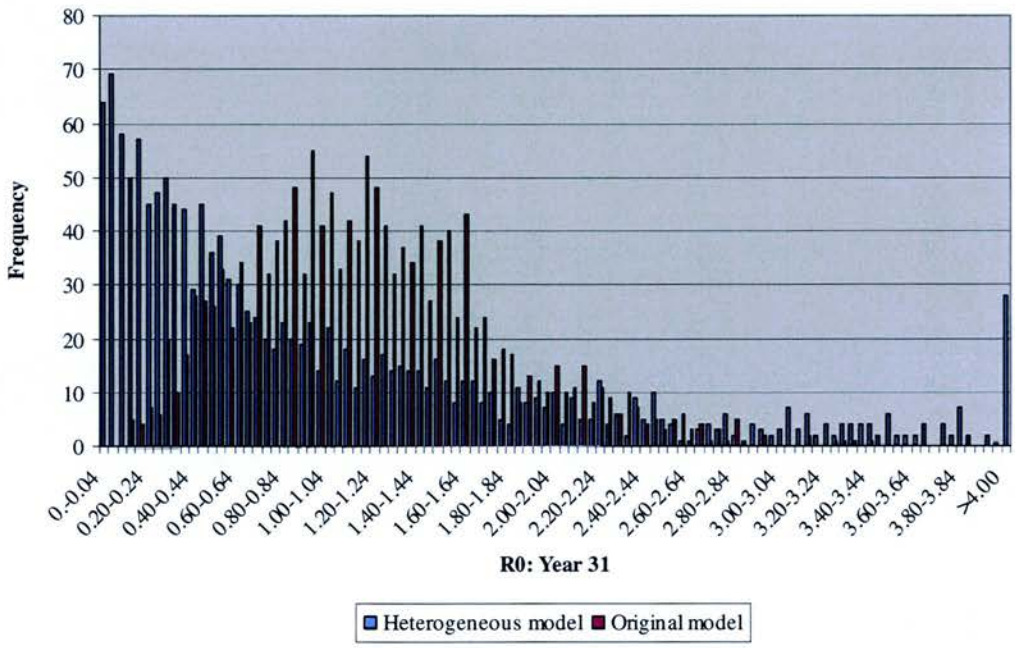

Frequency
70
60
50
40
30
20
10
0
0-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 26
Heterogeneous model
Original model

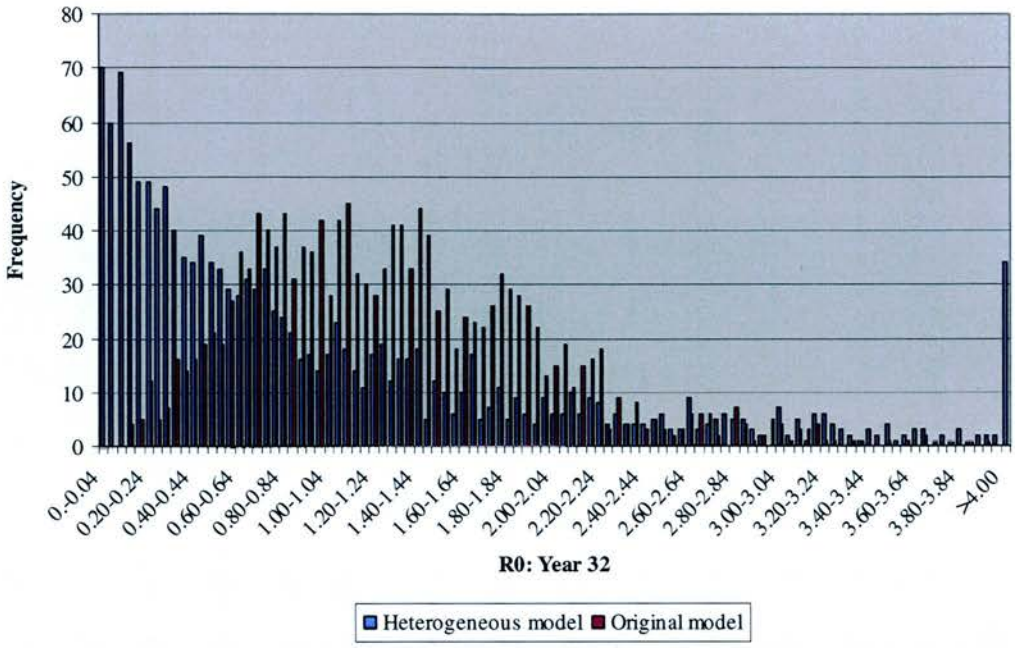

Frequency
80
70
60
50
40
30
20
10
0
0-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 27
Heterogeneous model
Original model

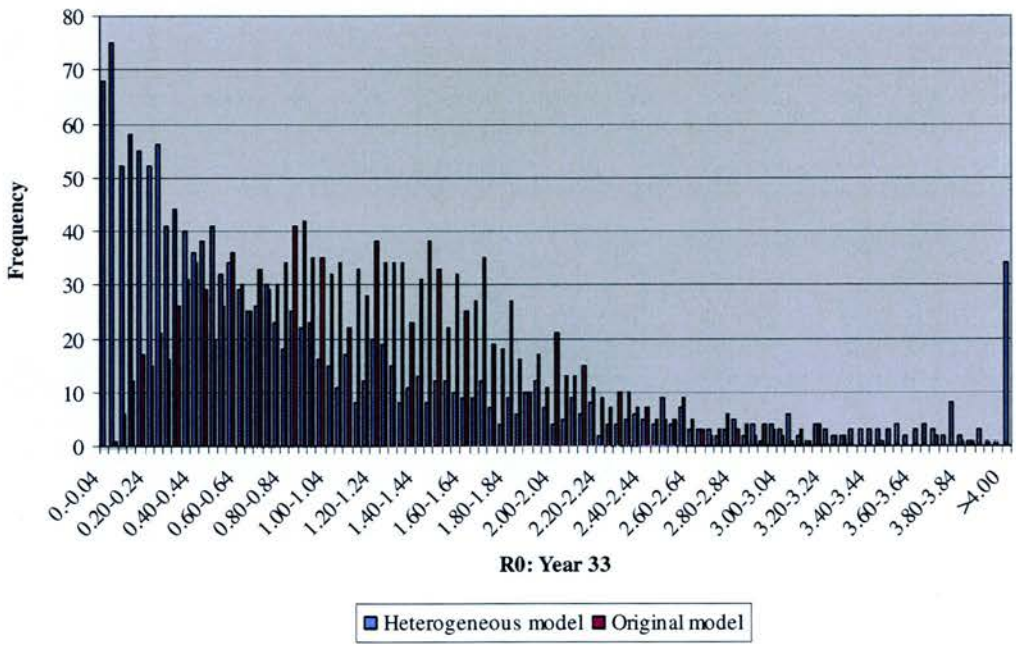

Frequency
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 28
Heterogeneous model
Original model

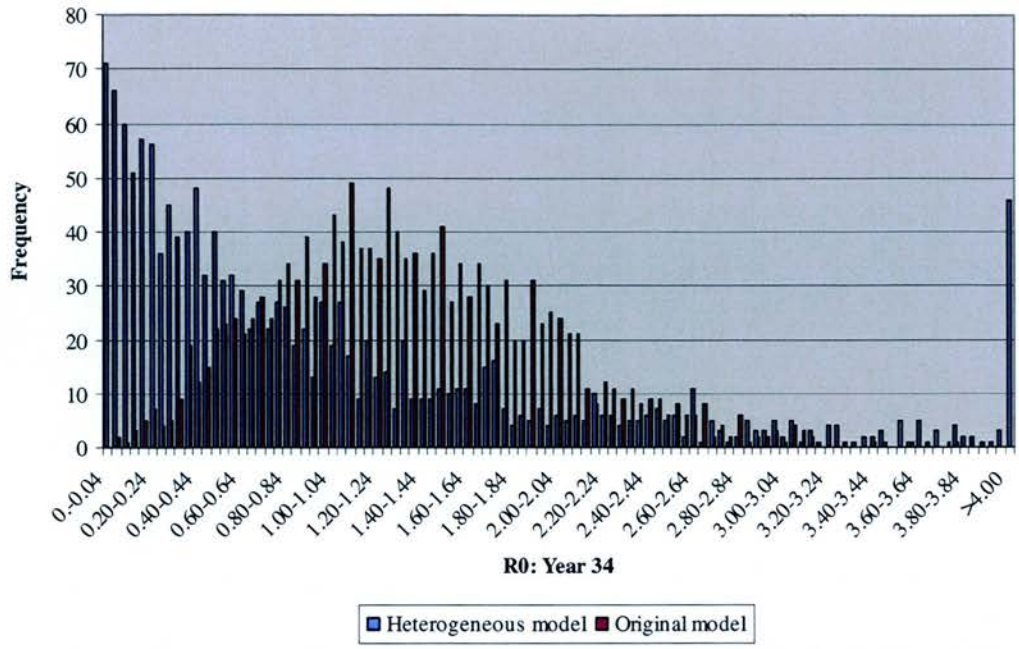

Frequency
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 29
Heterogeneous model
Original model

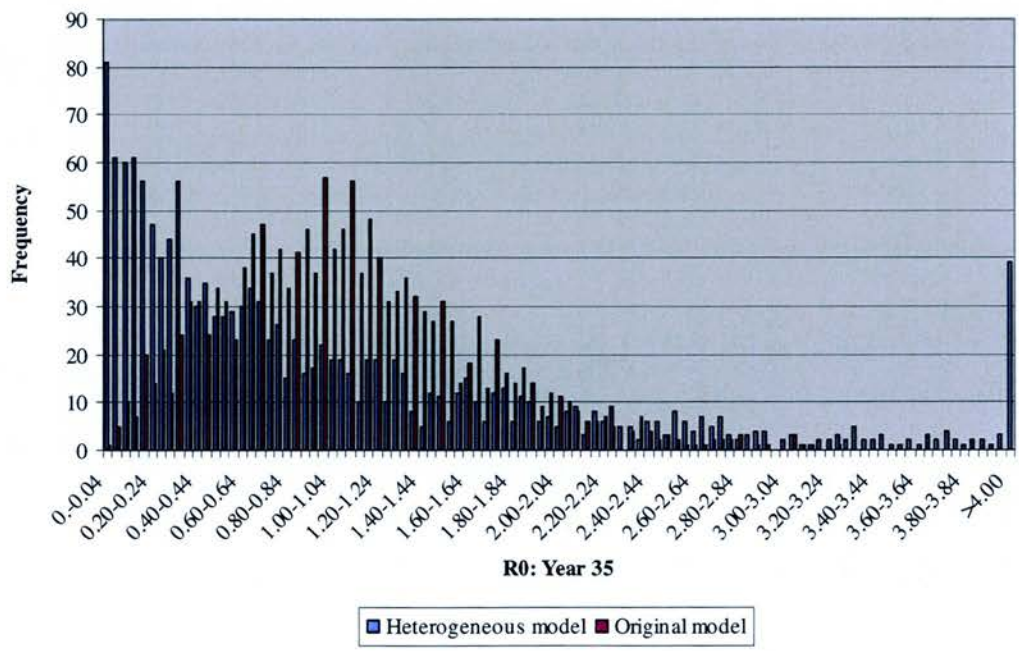

Frequency
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 30
Heterogeneous model
Original model


Frequency
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 31
Heterogeneous model
Original model

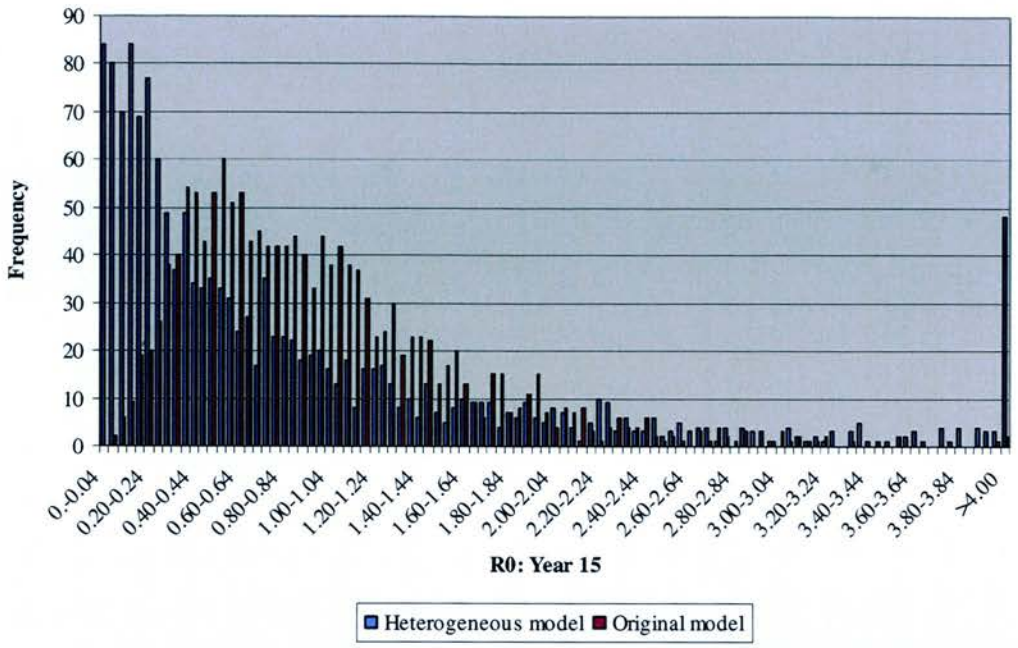

Frequency
80
70
60
50
40
30
20
10
0
0-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 32
Heterogeneous model
Original model

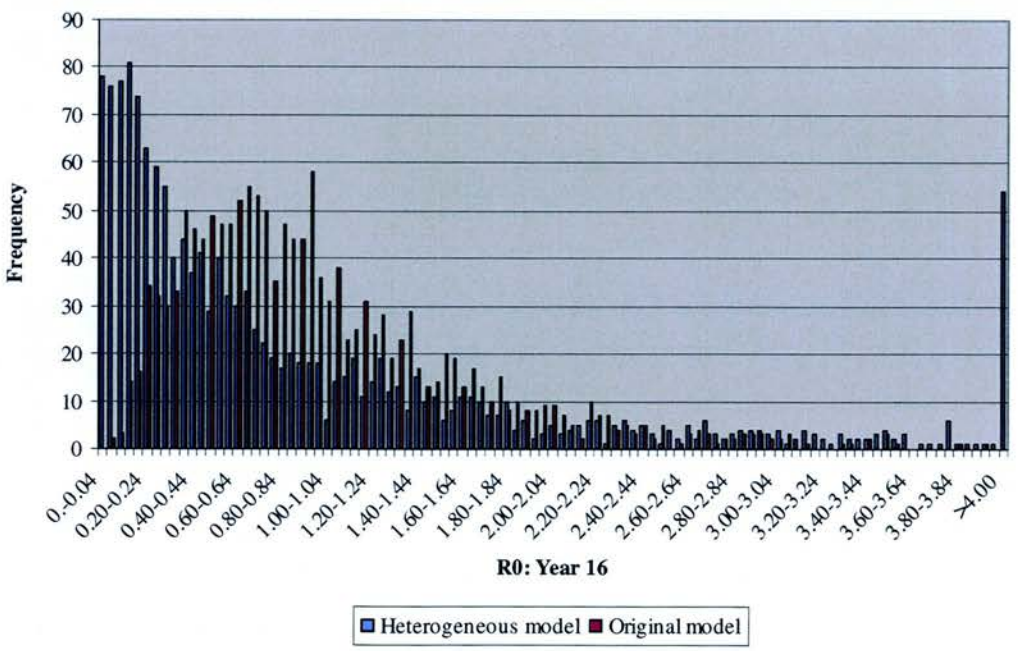

Frequency
80
70
60
50
40
30
20
10
0
0-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 33
Heterogeneous model
Original model

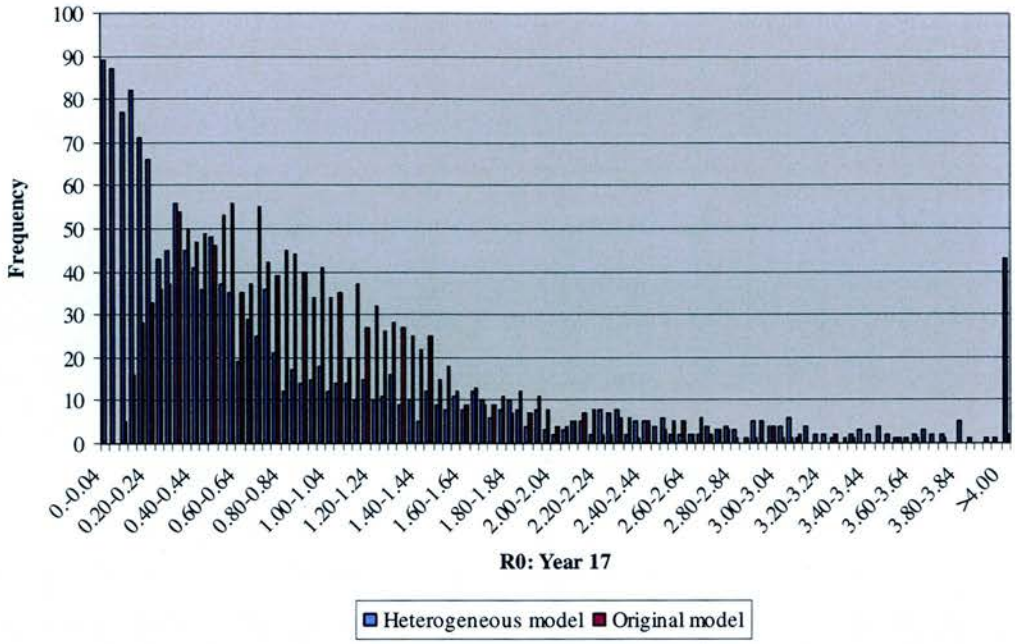

Frequency
80
70
60
50
40
30
20
10
0
0-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 34
Heterogeneous model
Original model

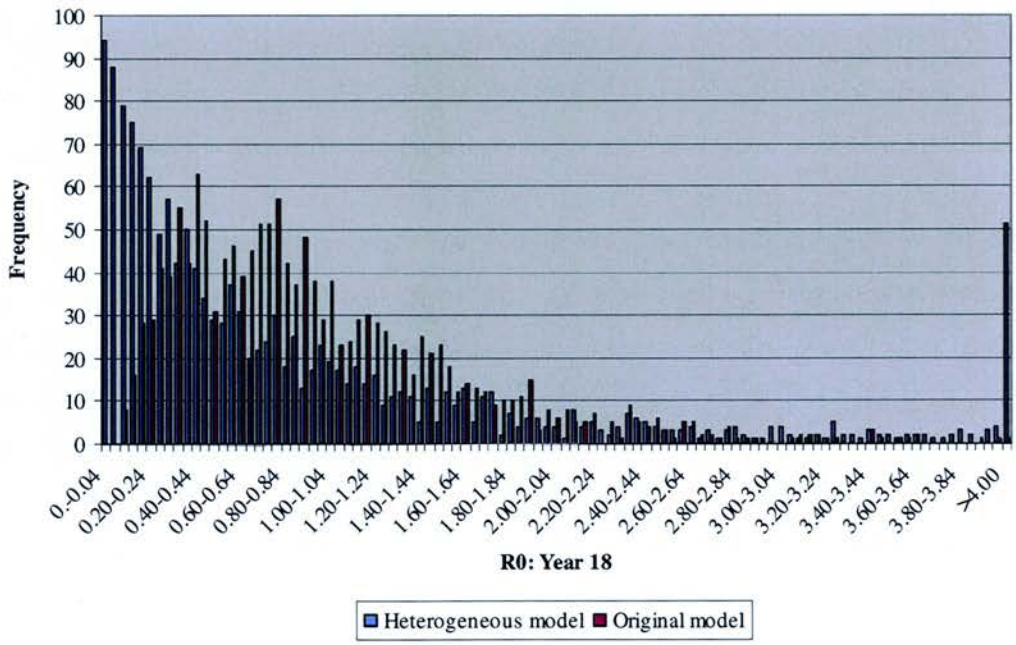

Frequency
90
80
70
60
50
40
30
20
10
0
0-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 35
Heterogeneous model
Original model

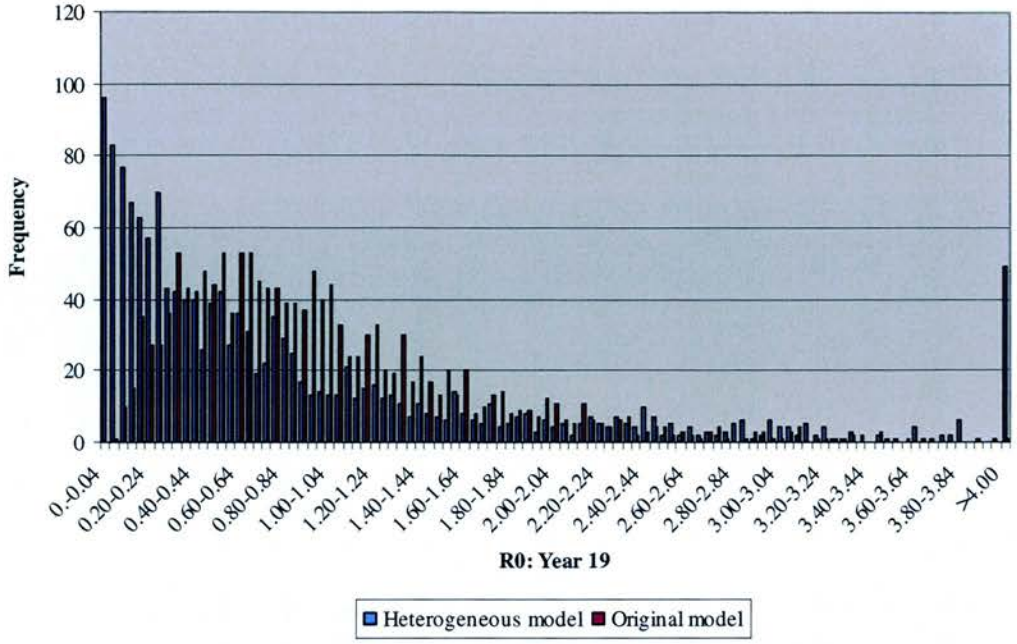
7.2 Texel

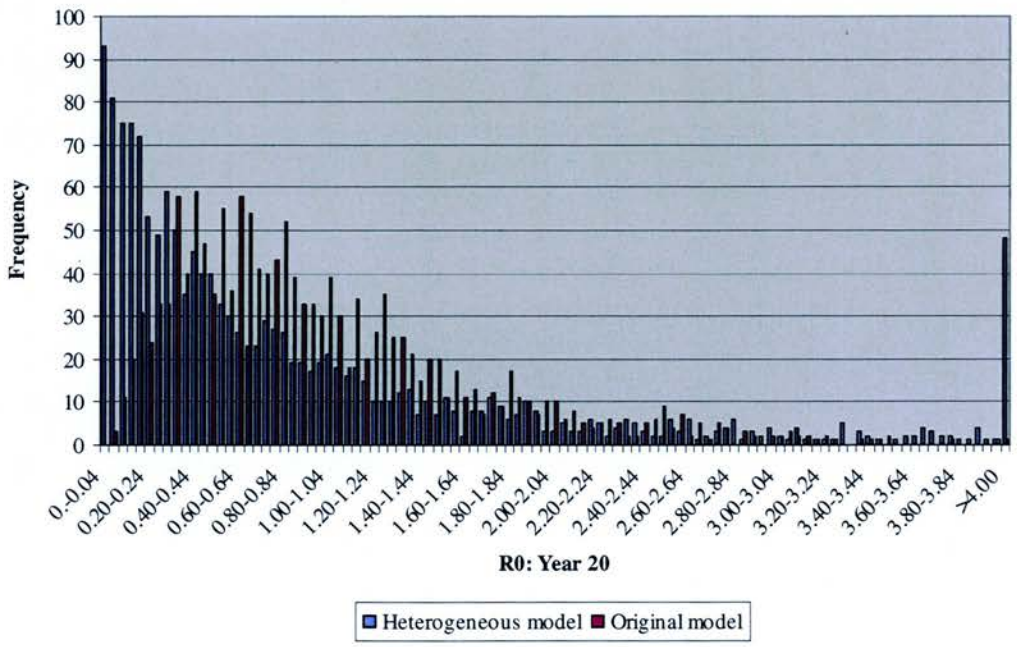

Frequency
90
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 15
Heterogeneous model
Original model

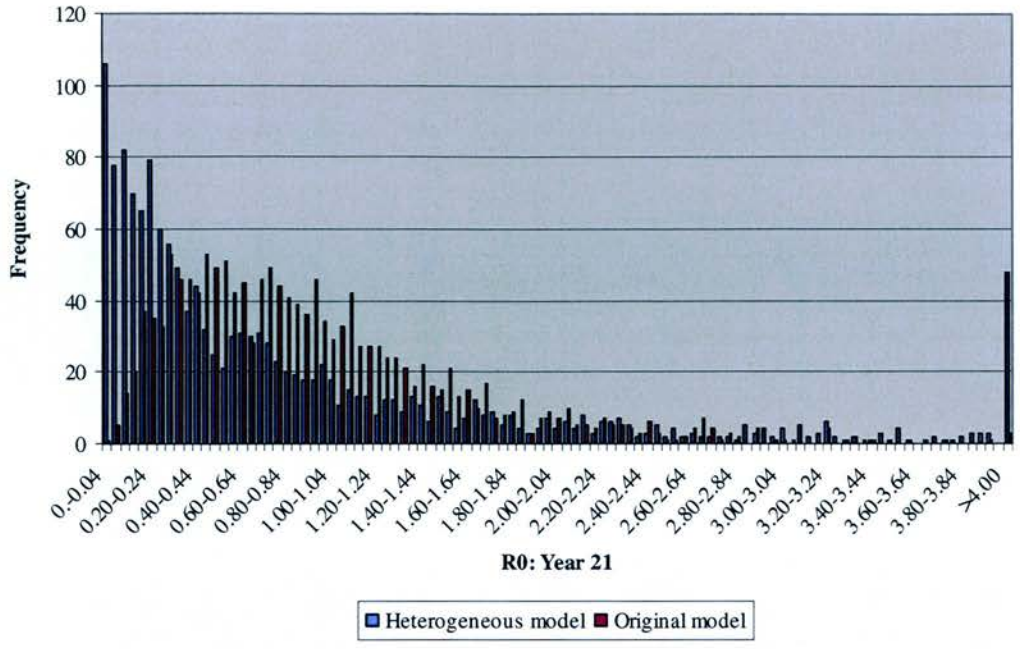

Frequency
90
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 16
Heterogeneous model
Original model

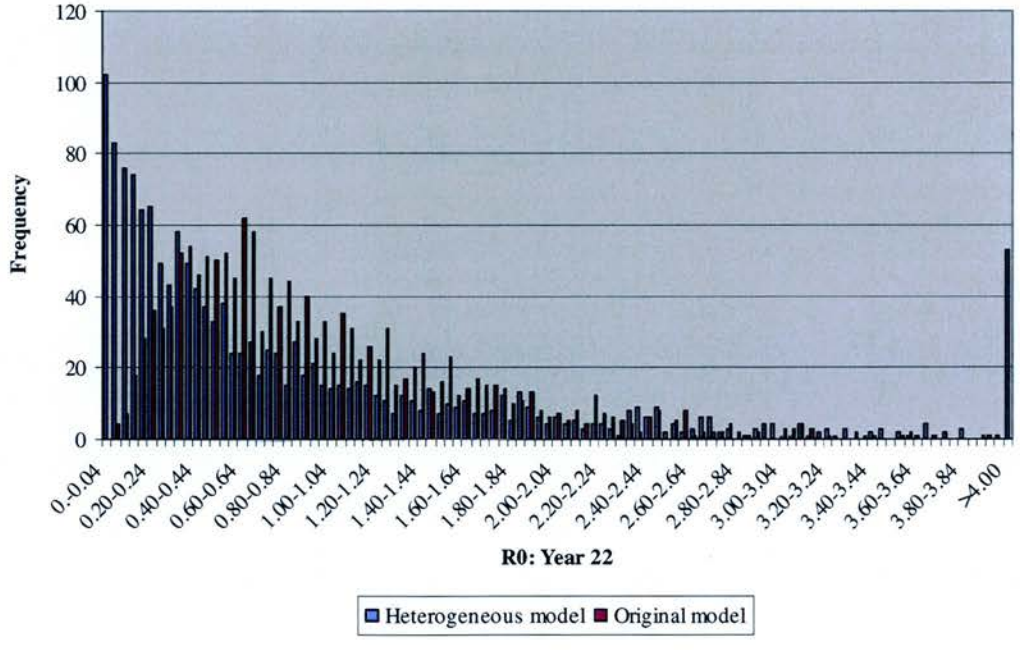

Frequency
100
90
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 17
Heterogeneous model
Original model

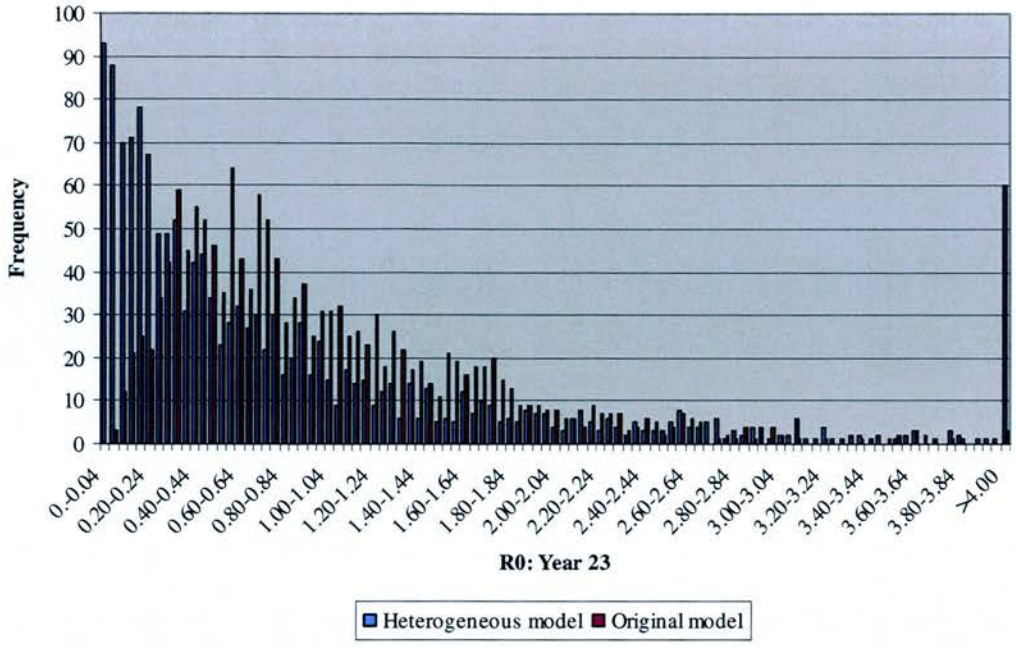

Frequency
100
90
80
70
60
50
40
30
20
10
0
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 18
Heterogeneous model
Original model

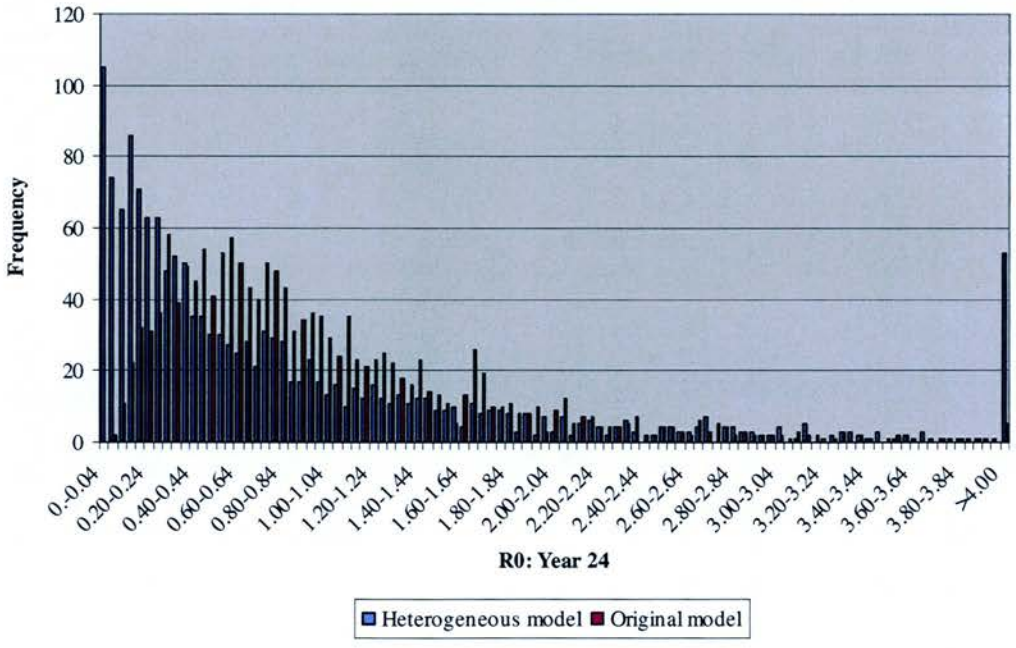

120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 19
Heterogeneous model
Original model


100
90
80
70
60
50
40
30
20
10
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 20
Heterogeneous model
Original model


120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 21
Heterogeneous model
Original model


120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 22
Heterogeneous model
Original model


Frequency
100
90
80
70
60
50
40
30
20
10
0
0,-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 23
Heterogeneous model
Original model


Frequency
120
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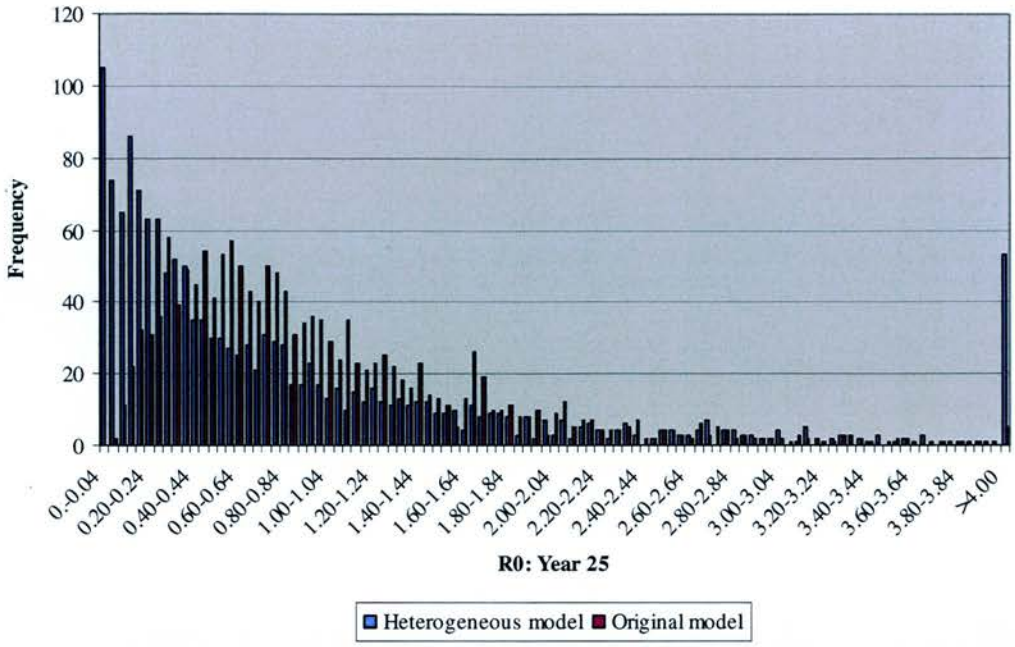
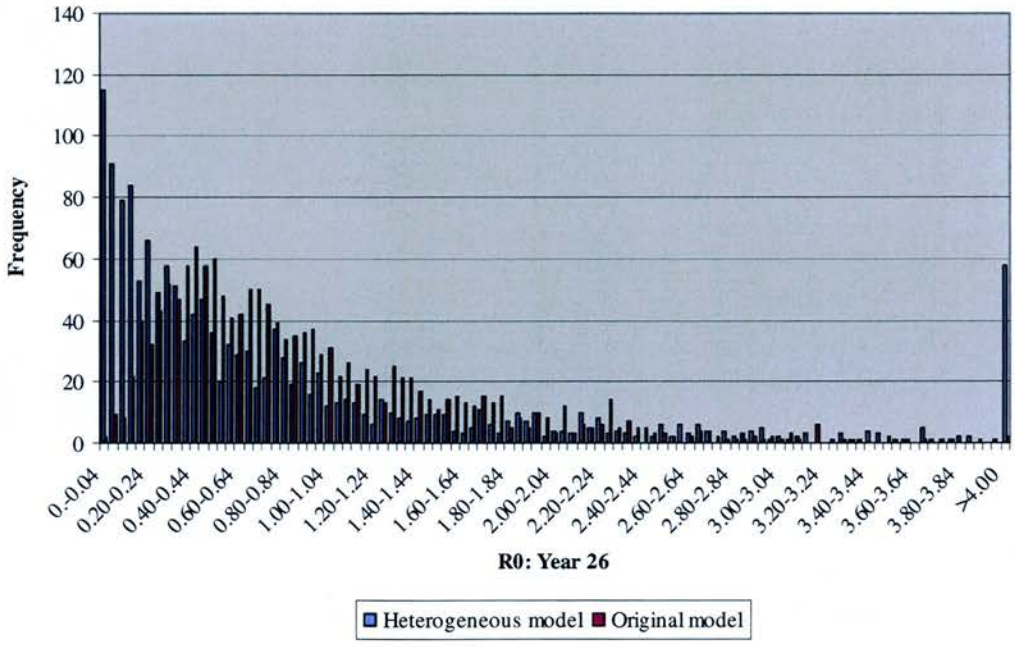
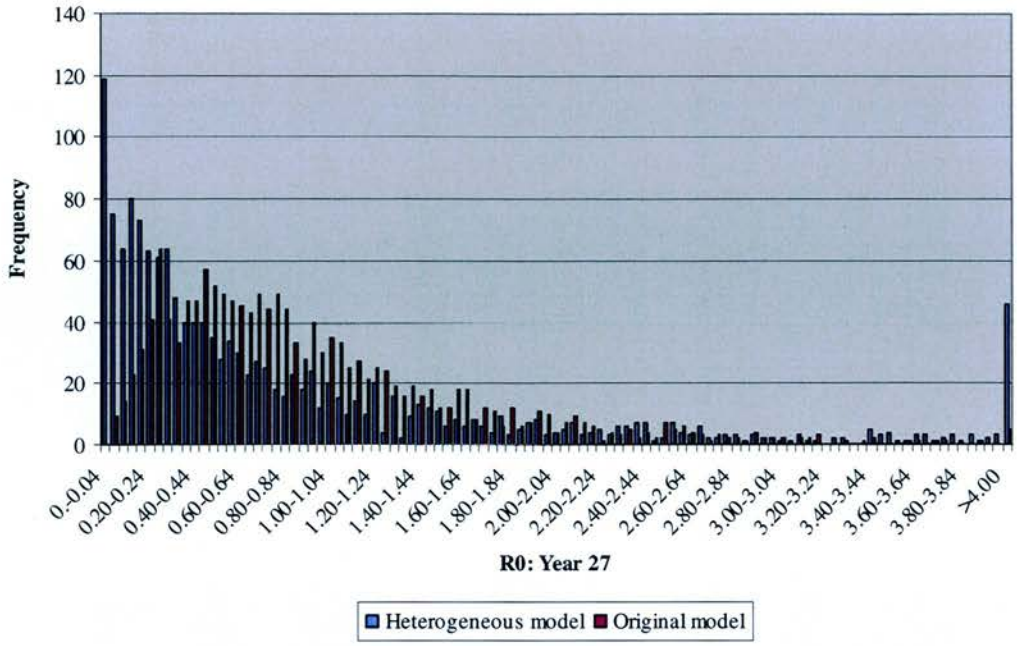
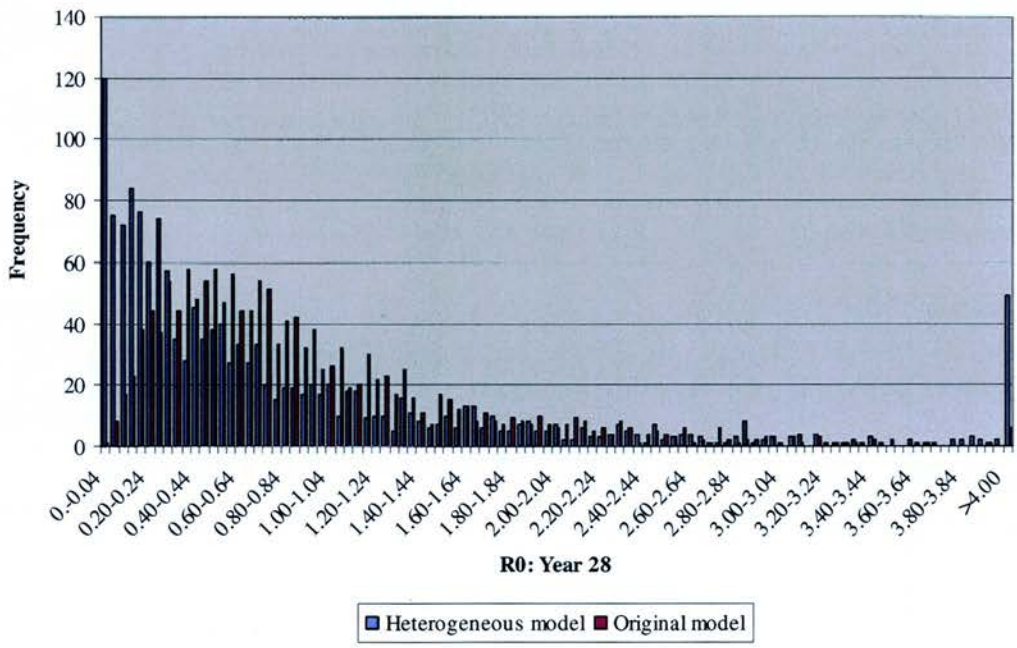
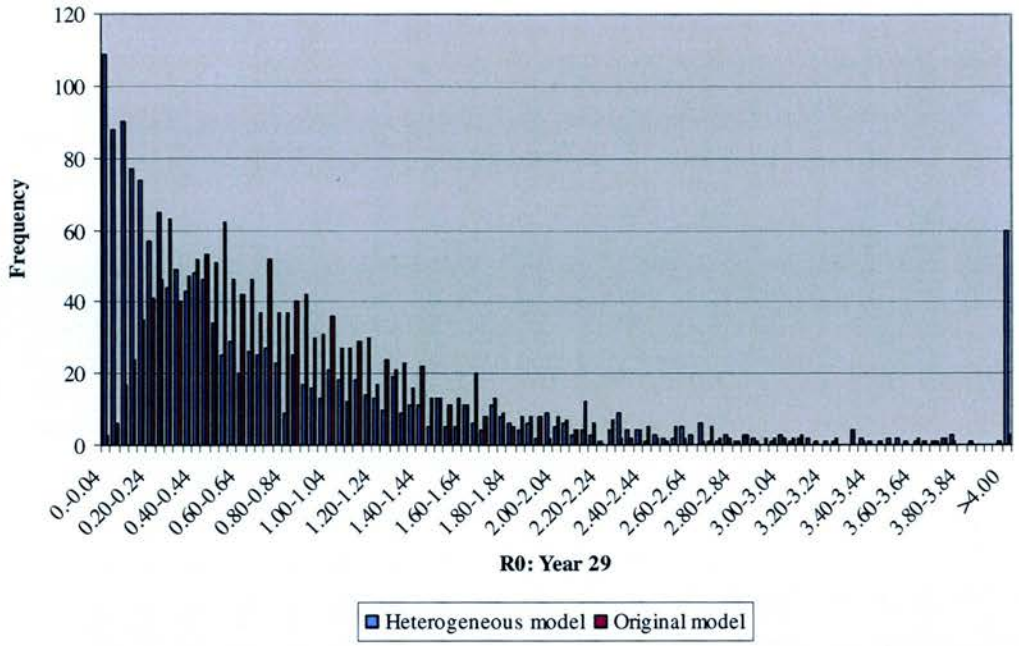
80
60
40
20
0
0,-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 24
Heterogeneous model
Original model

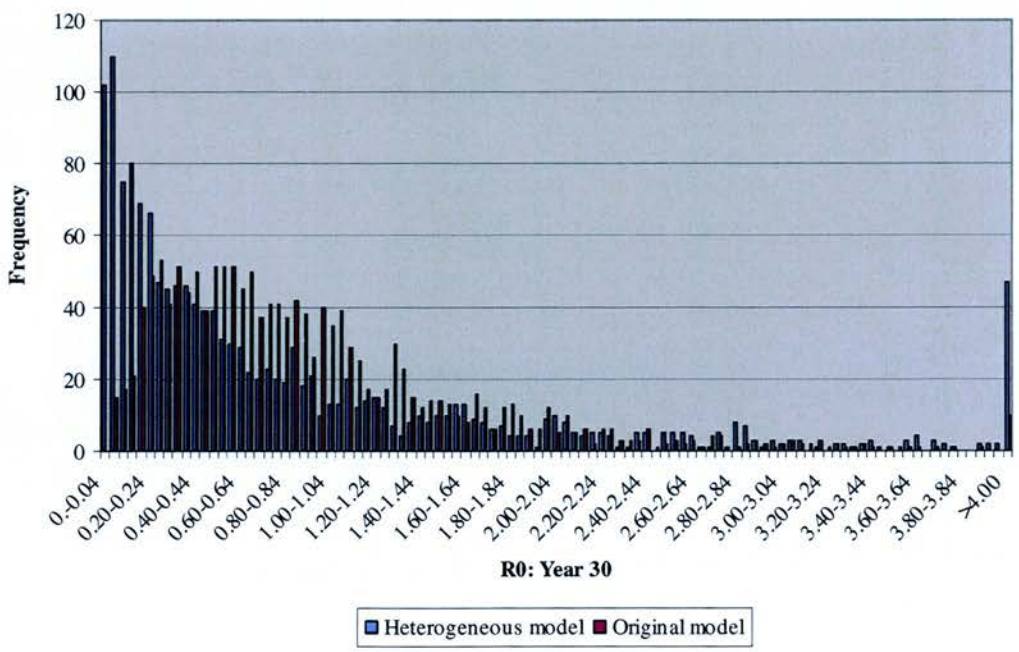

120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 25
Heterogeneous model
Original model

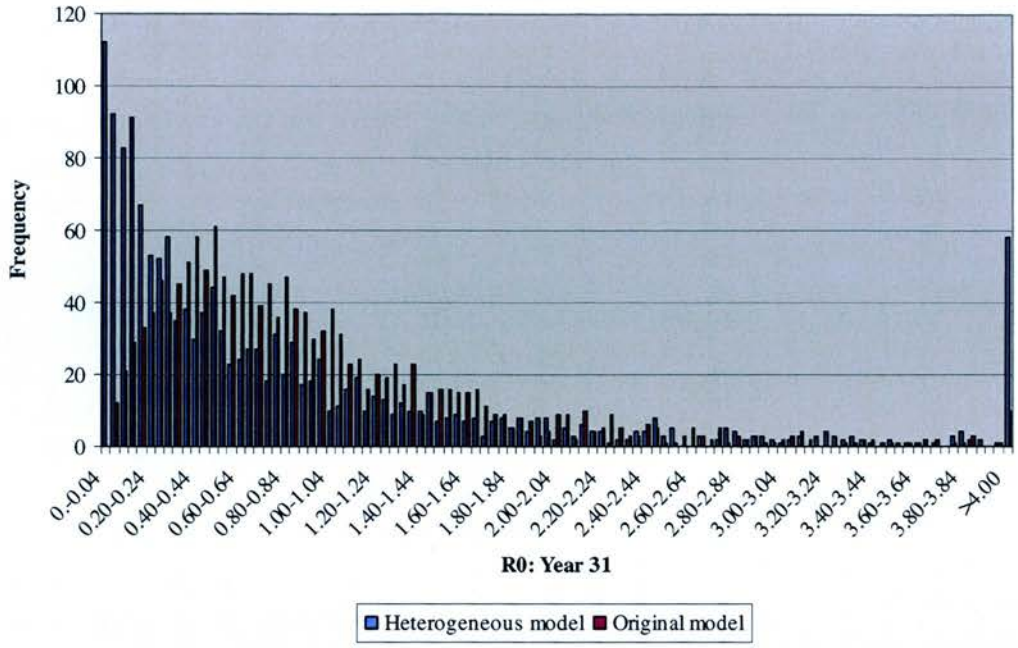

140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 26
Heterogeneous model
Original model

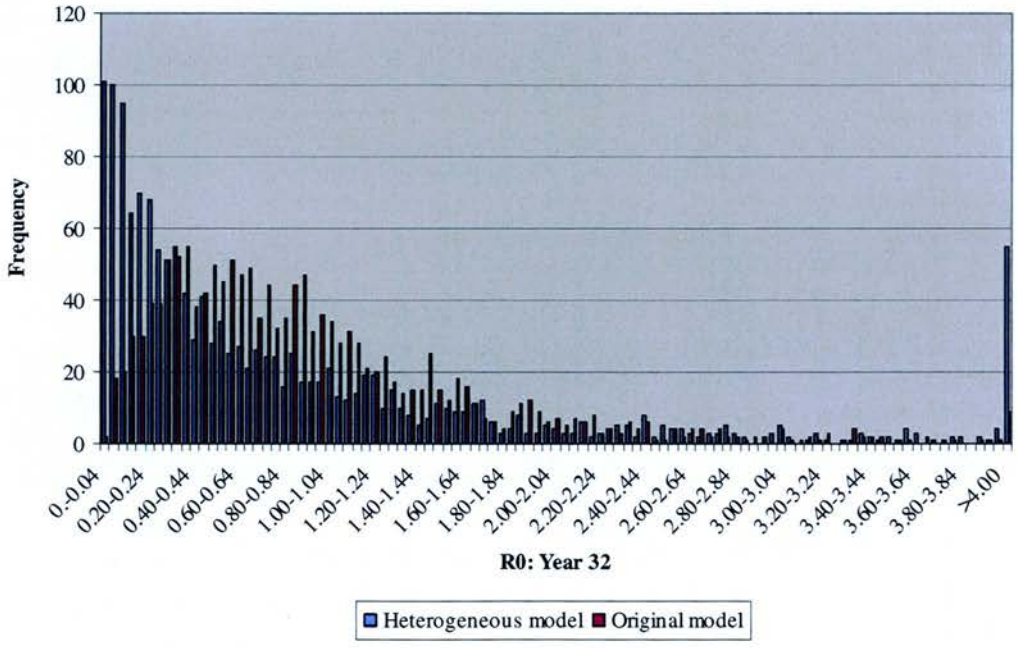

140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 27
Heterogeneous model
Original model

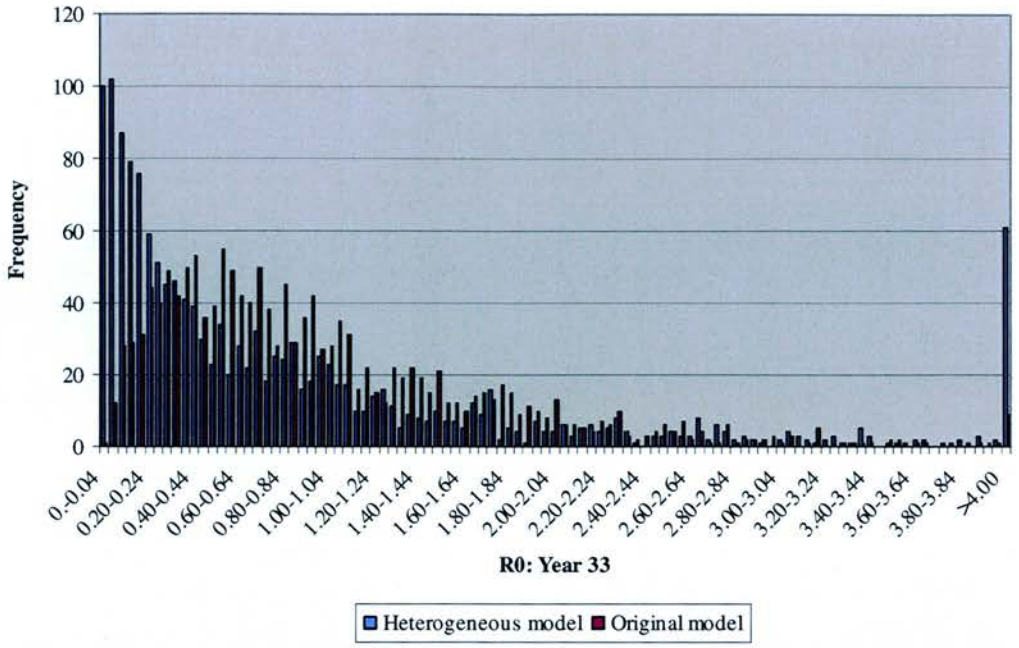

140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 28
Heterogeneous model
Original model

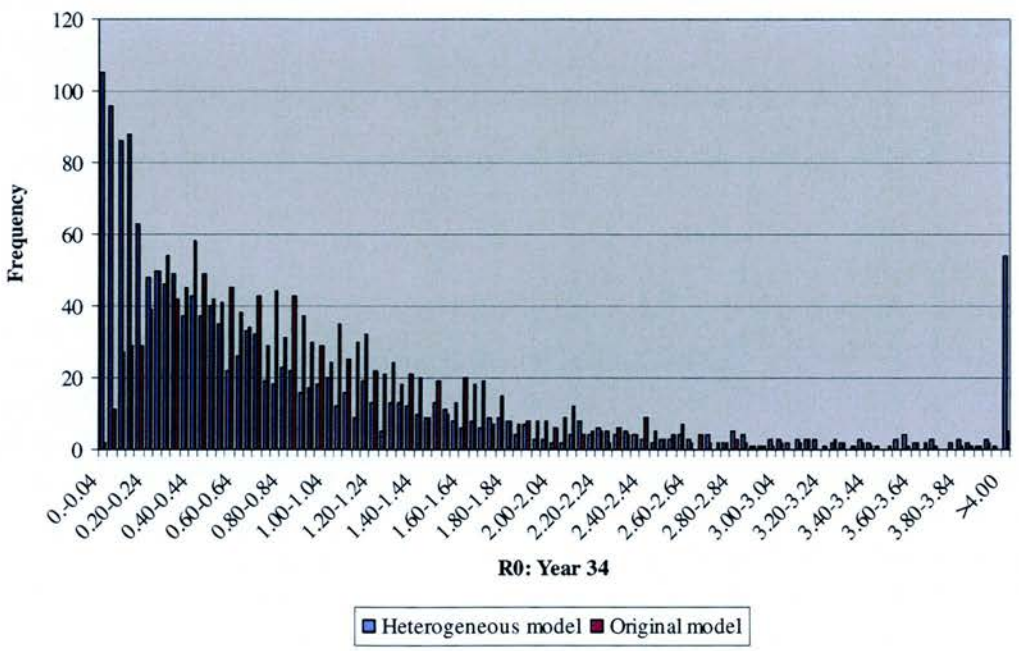

120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 29
Heterogeneous model
Original model

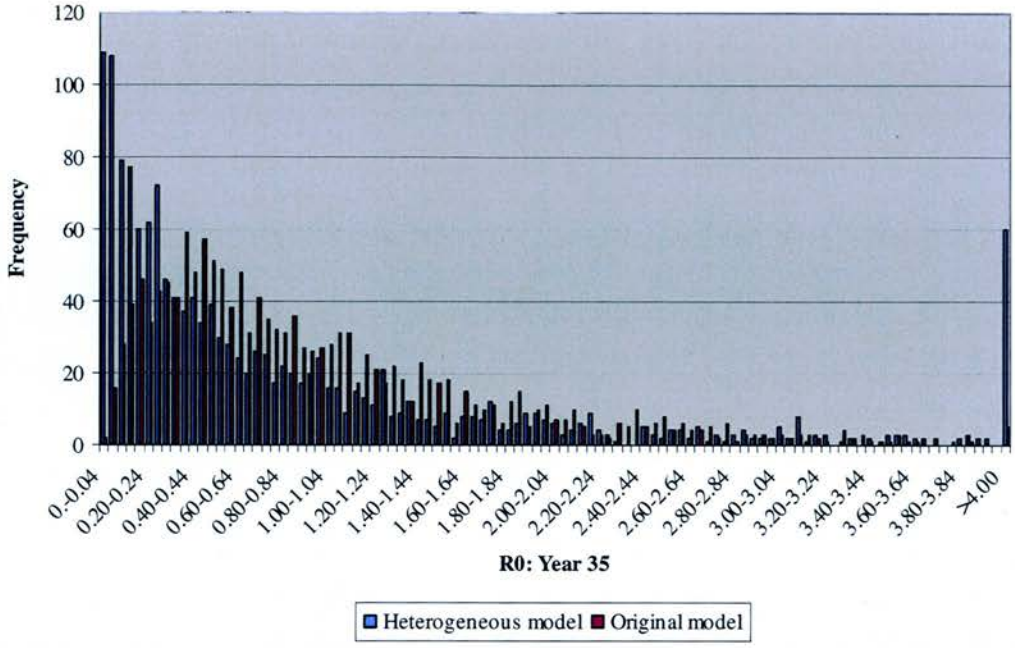

120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 30
Heterogeneous model
Original model


120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 31
Heterogeneous model
Original model

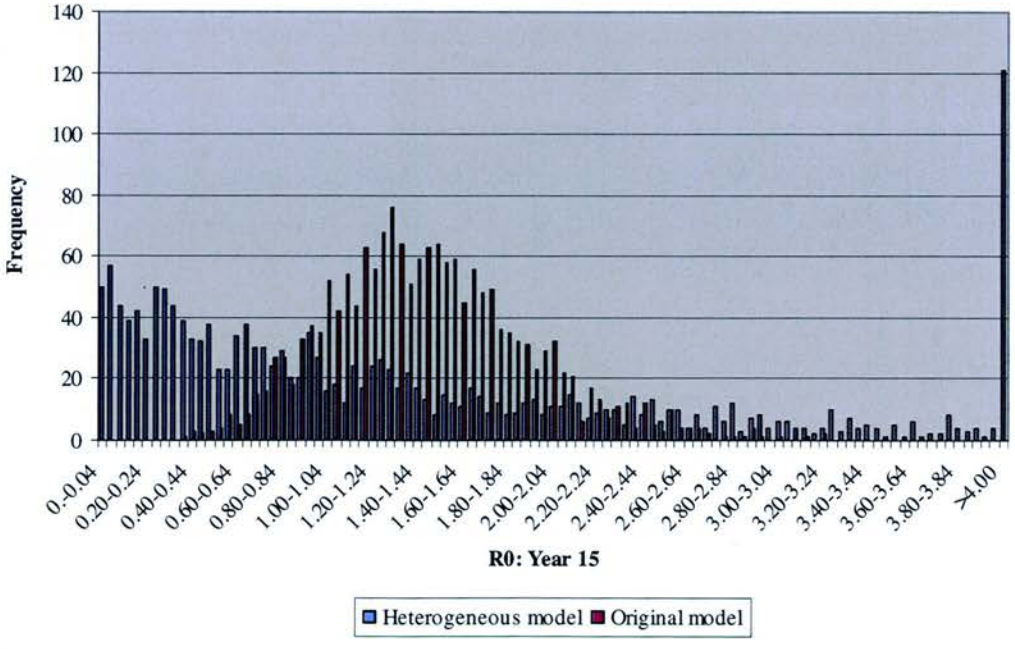

120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 32
Heterogeneous model
Original model

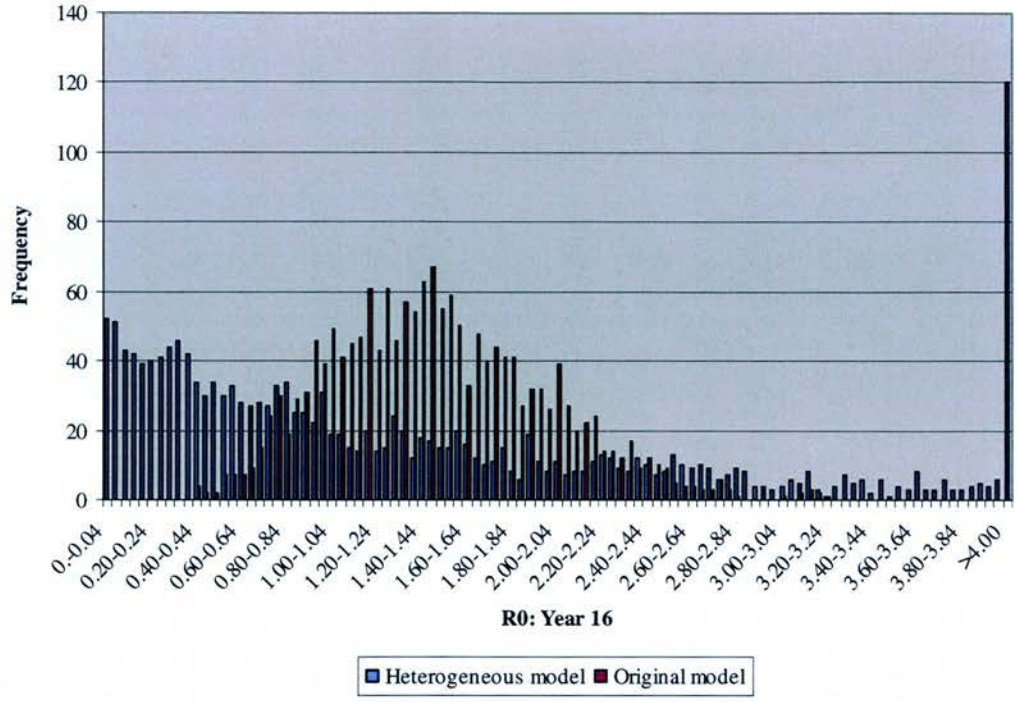

120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 33
Heterogeneous model
Original model

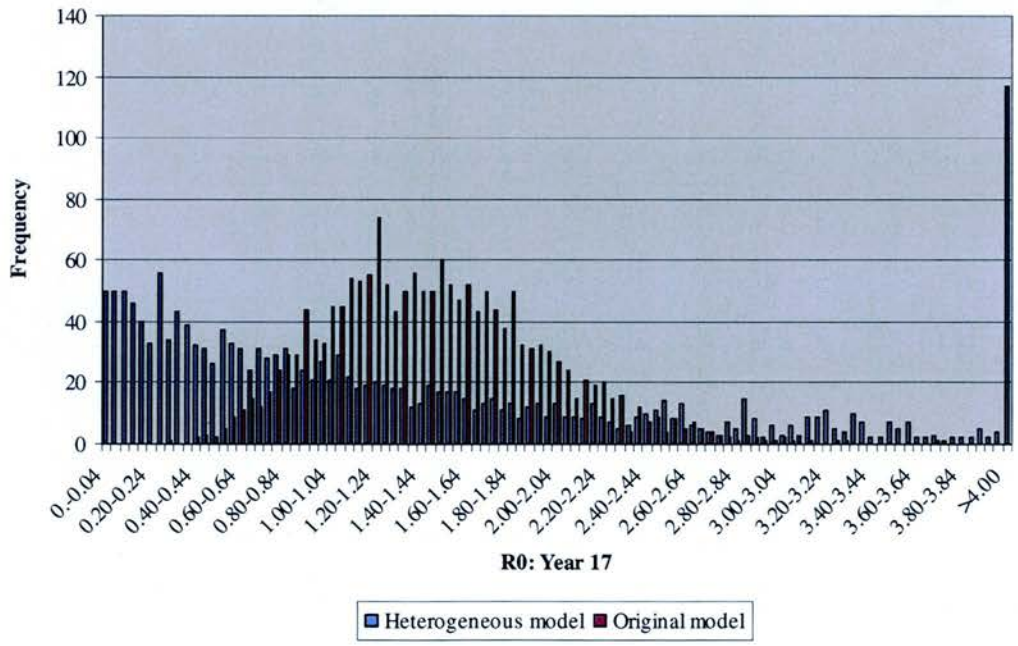

120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 34
Heterogeneous model
Original model

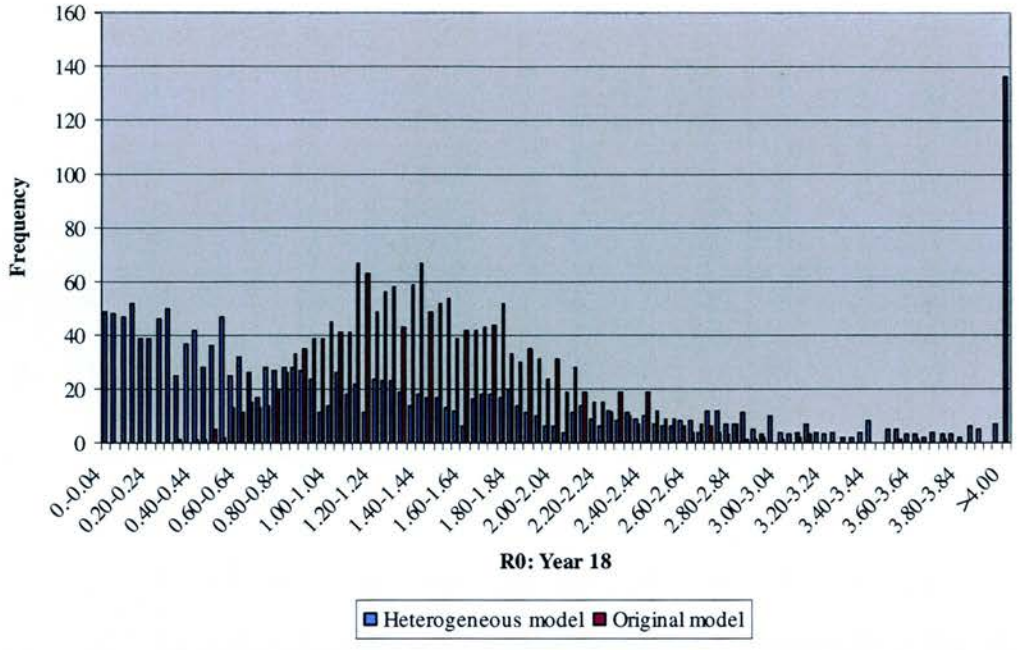


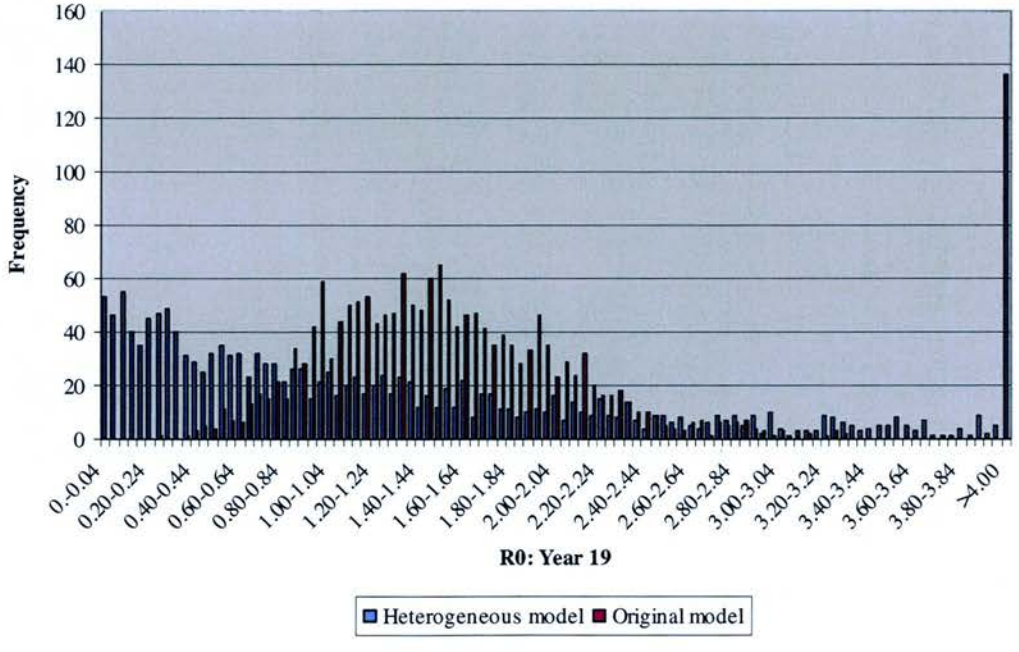
7.3 Swaledale

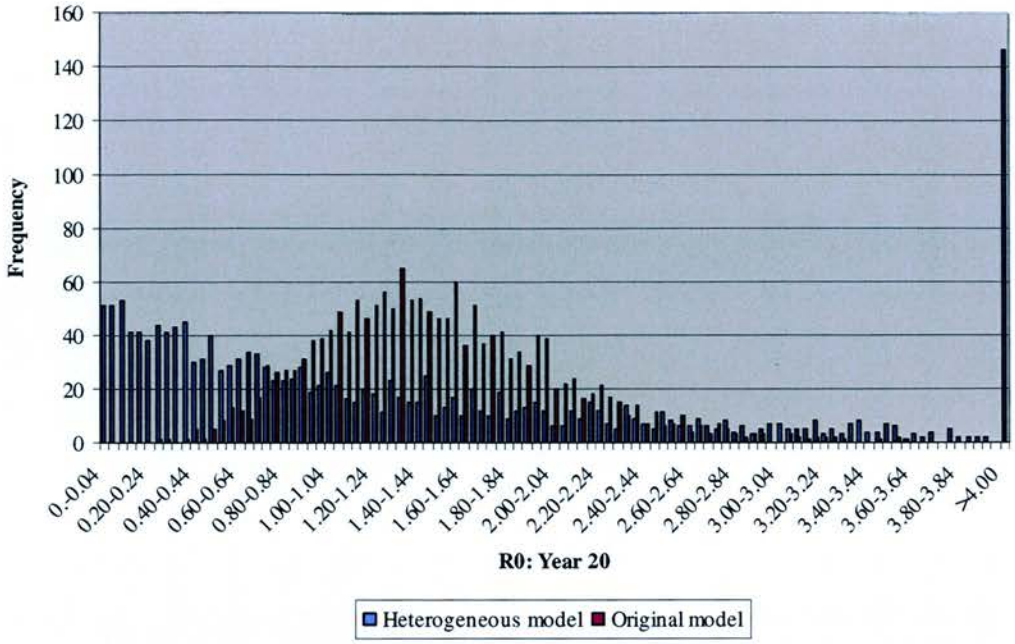


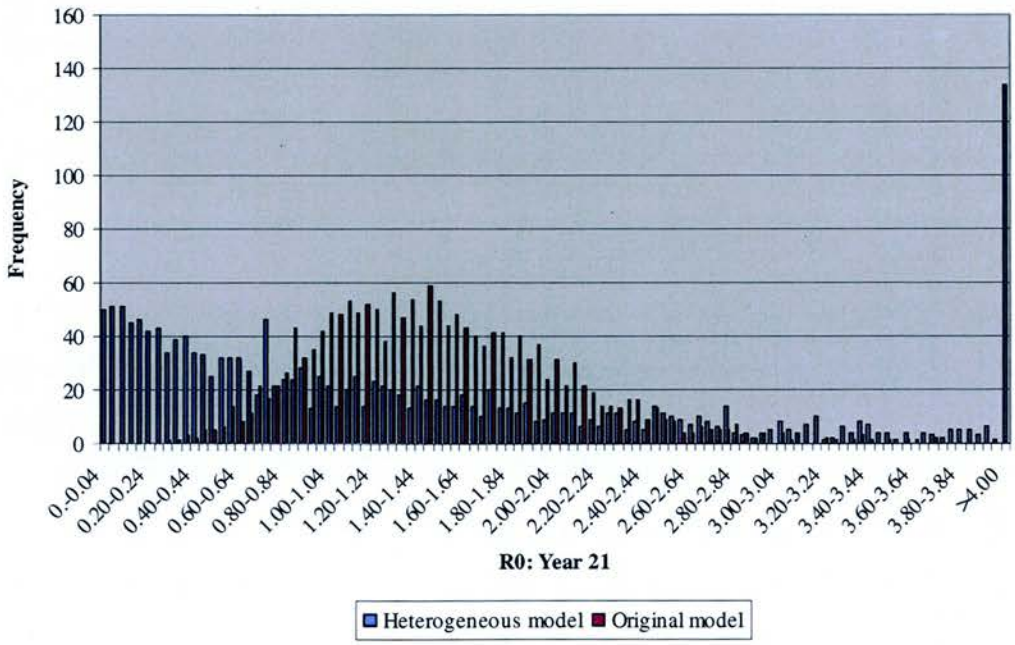

140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 16
Heterogeneous model
Original model

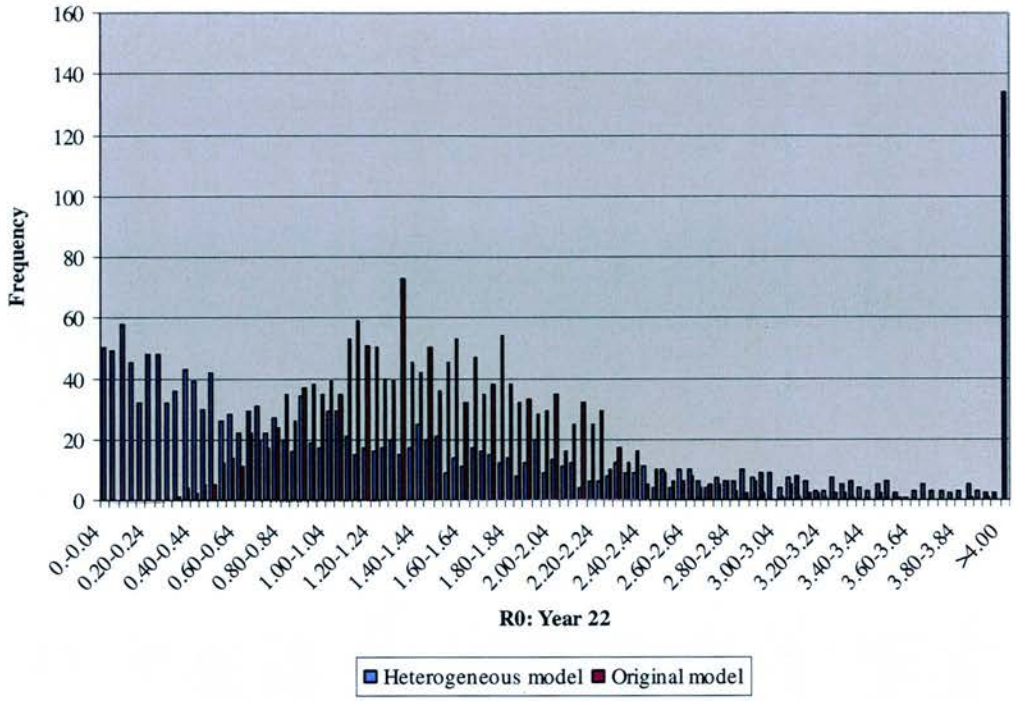

140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 17
Heterogeneous model
Original model

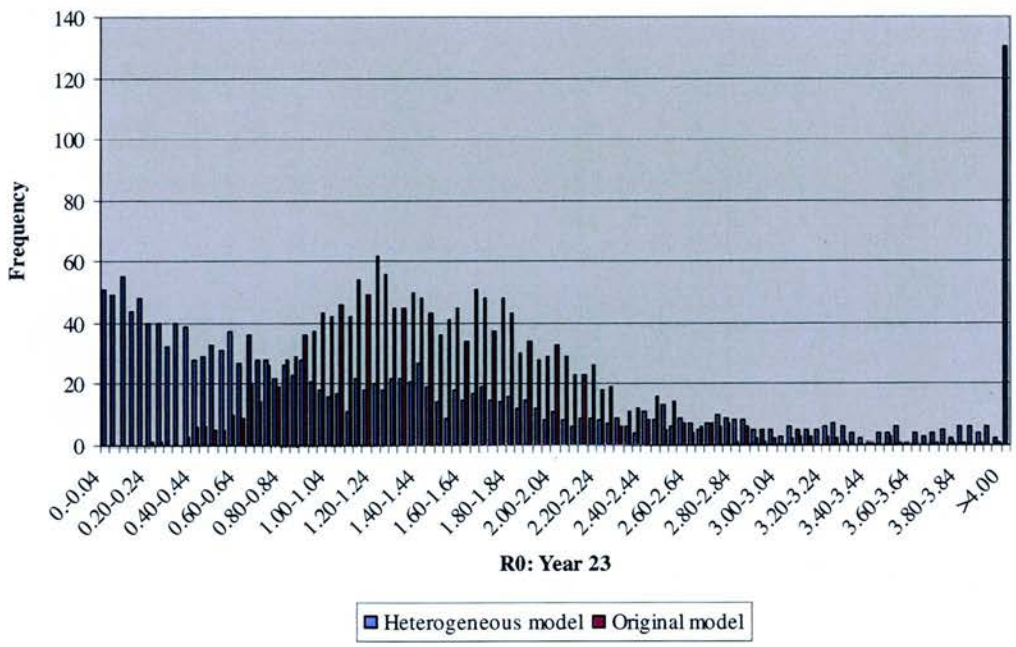

160
140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 18
Heterogeneous model
Original model

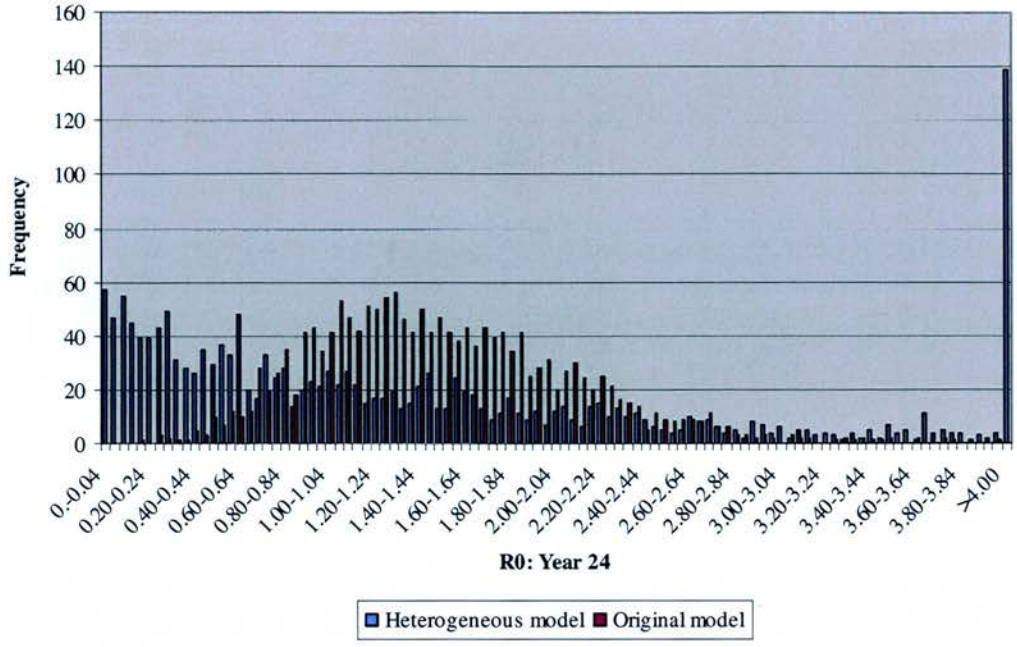

160
140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 19
Heterogeneous model
Original model


160
140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 20
Heterogeneous model
Original model


160
140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 21
Heterogeneous model
Original model


160
140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 22
Heterogeneous model
Original model


140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 23
Heterogeneous model
Original model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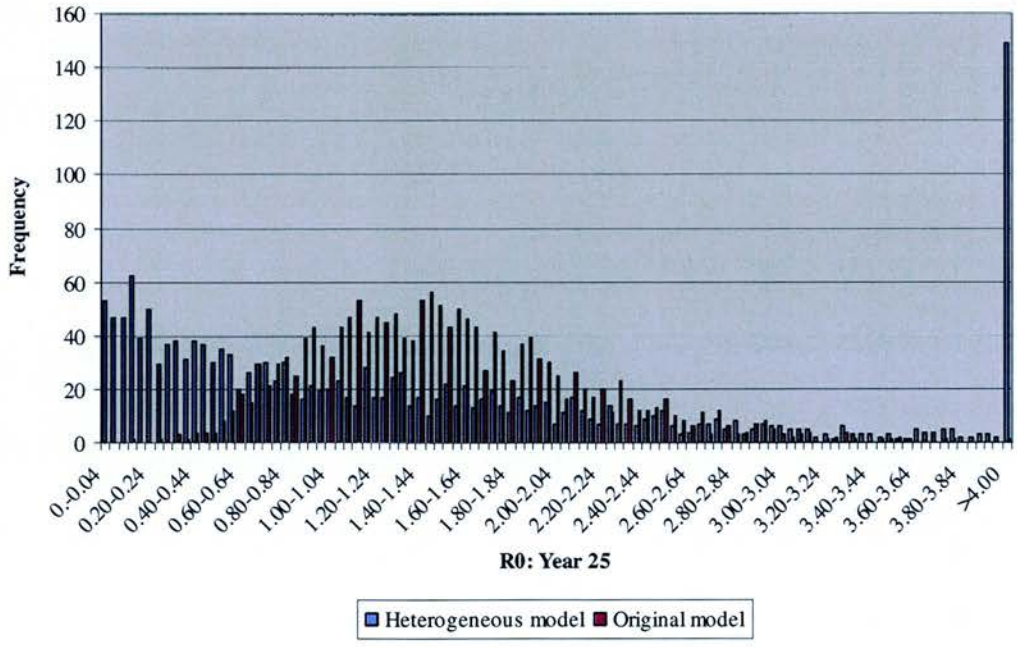
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 24
Heterogeneous model
Original model


160
140
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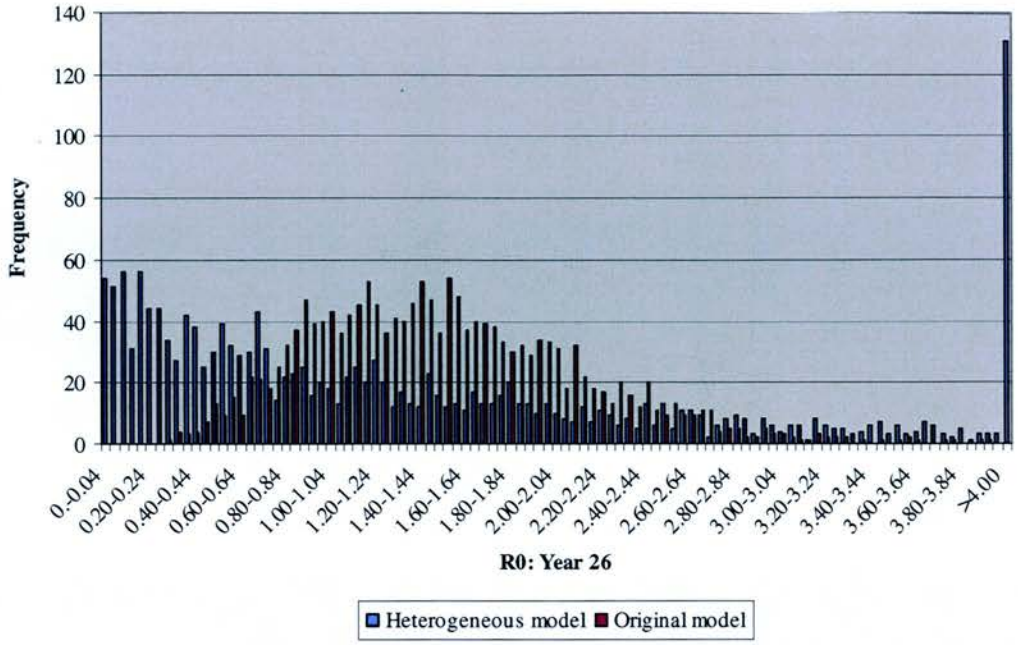
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 25
Heterogeneous model
Original model


140
120
100
80
60
40
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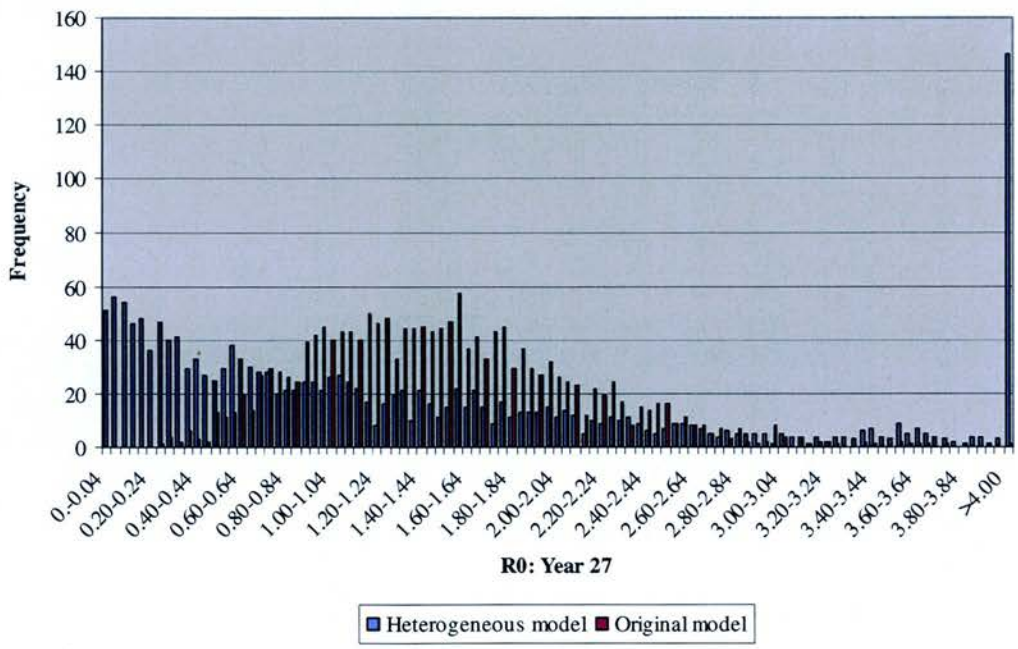
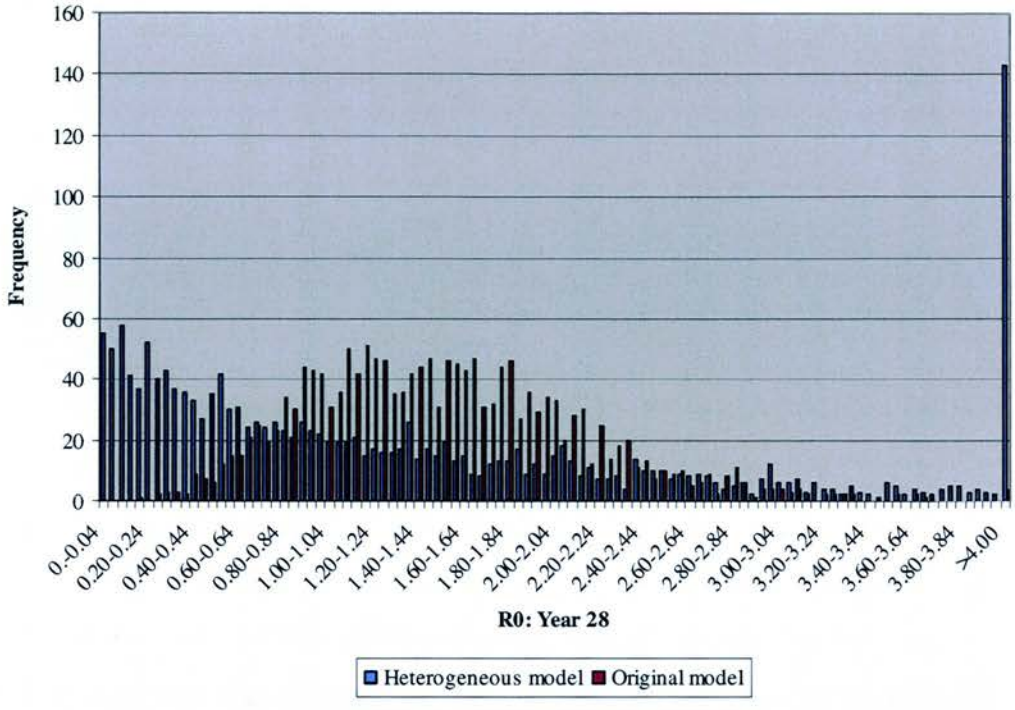
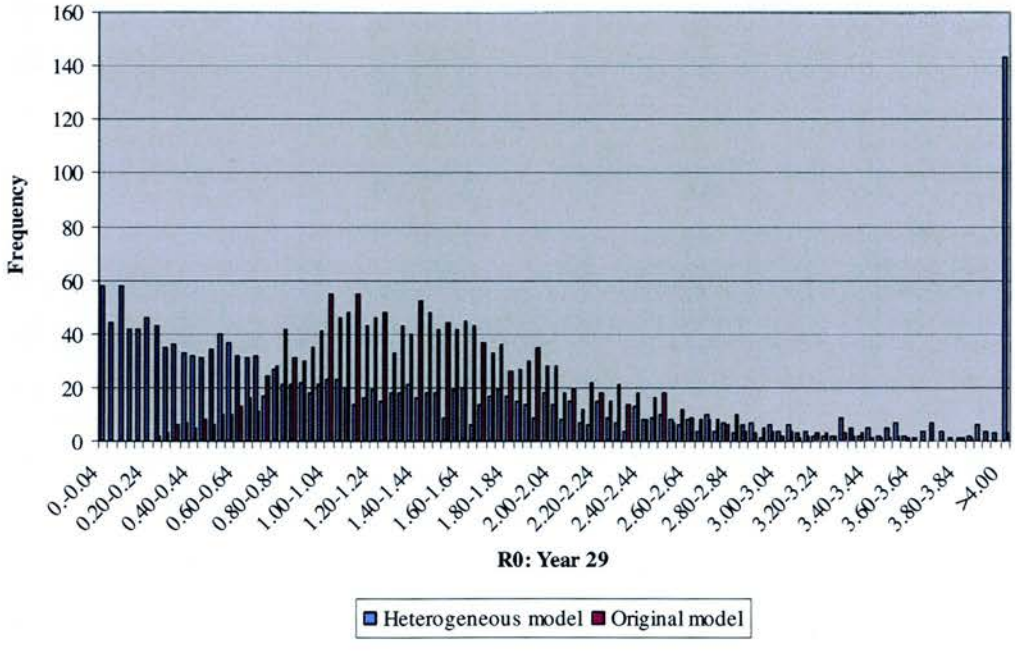
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 26
Heterogeneous model
Original model


160
140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 27
Heterogeneous model
Original model


160
140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 28
Heterogeneous model
Original model


160
140
120
100
80
60
40
20
0
Frequency
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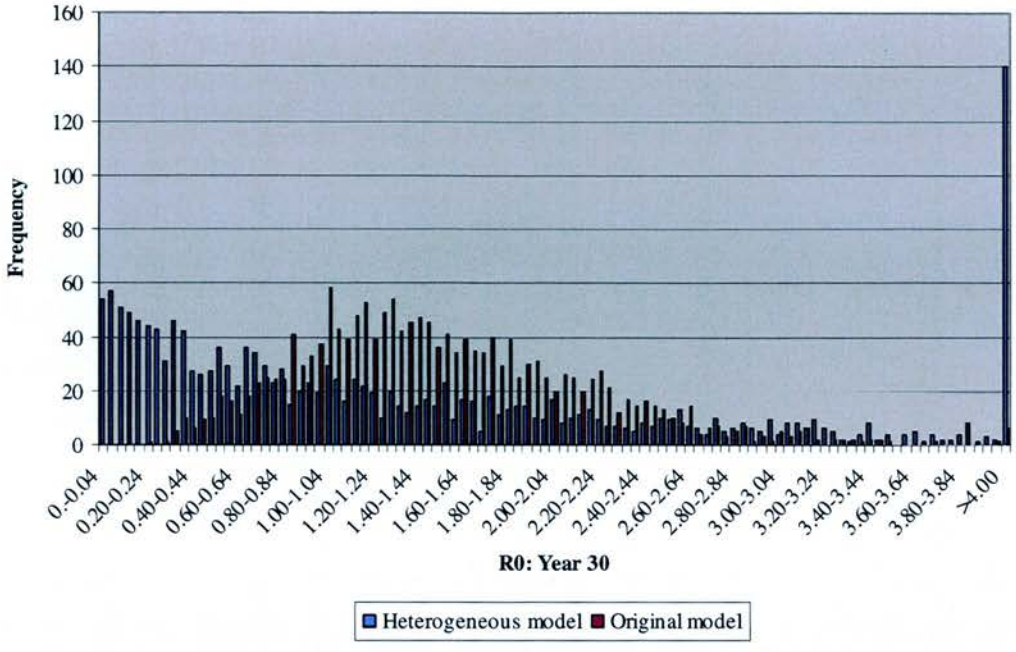
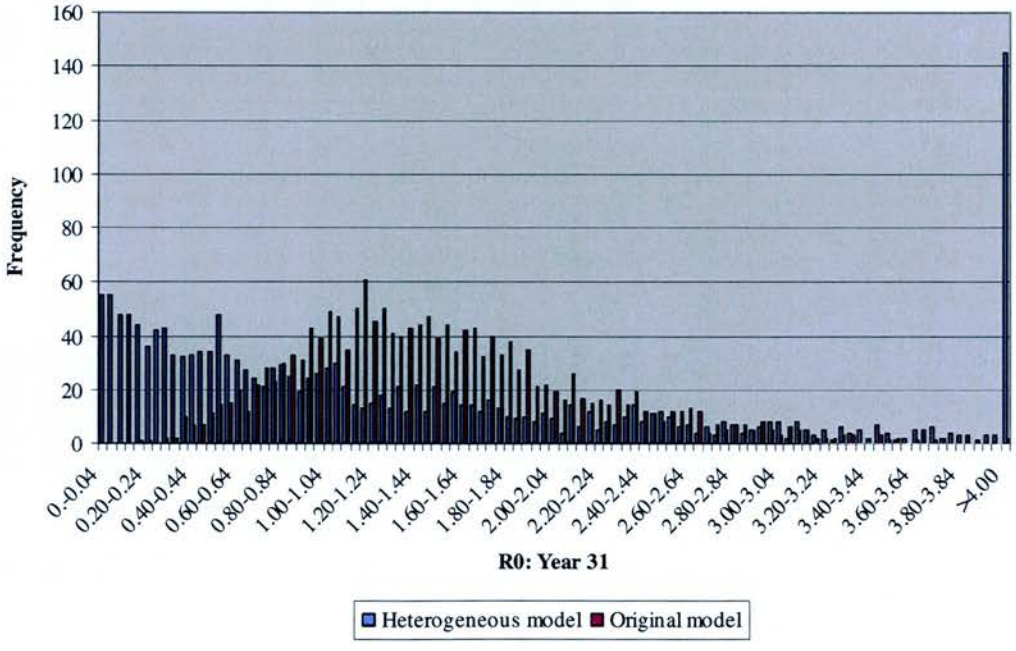
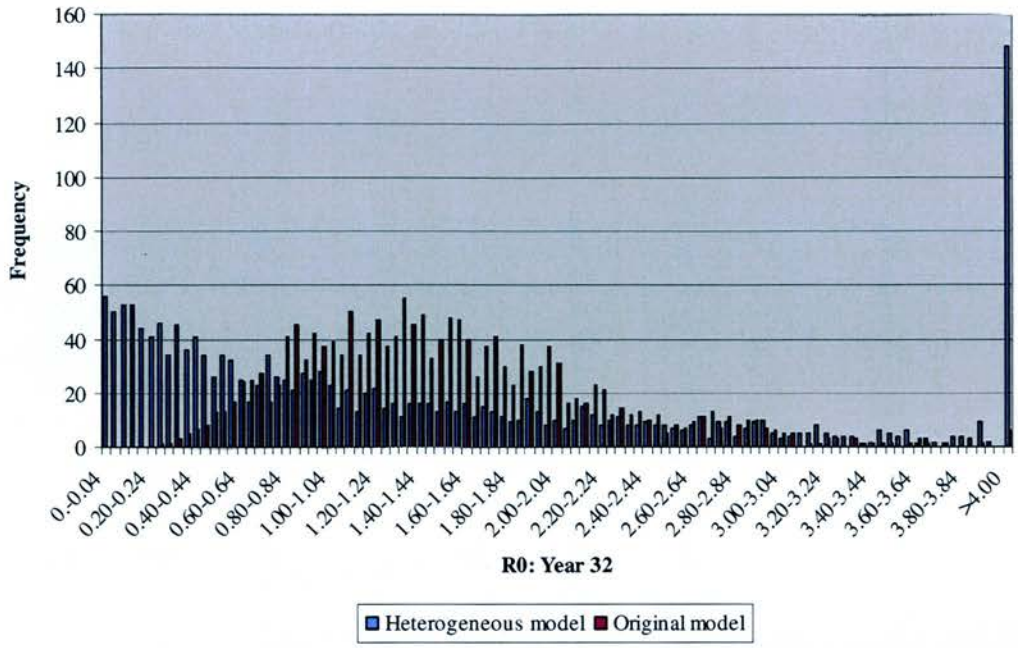
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 29
Heterogeneous model
Original model


160
140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 30
Heterogeneous model
Original model


160
140
120
100
80
60
40
20
0
Frequency
0.-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 31
Heterogeneous model
Original model


160
140
120
100
80
60
40
20
0
Frequency
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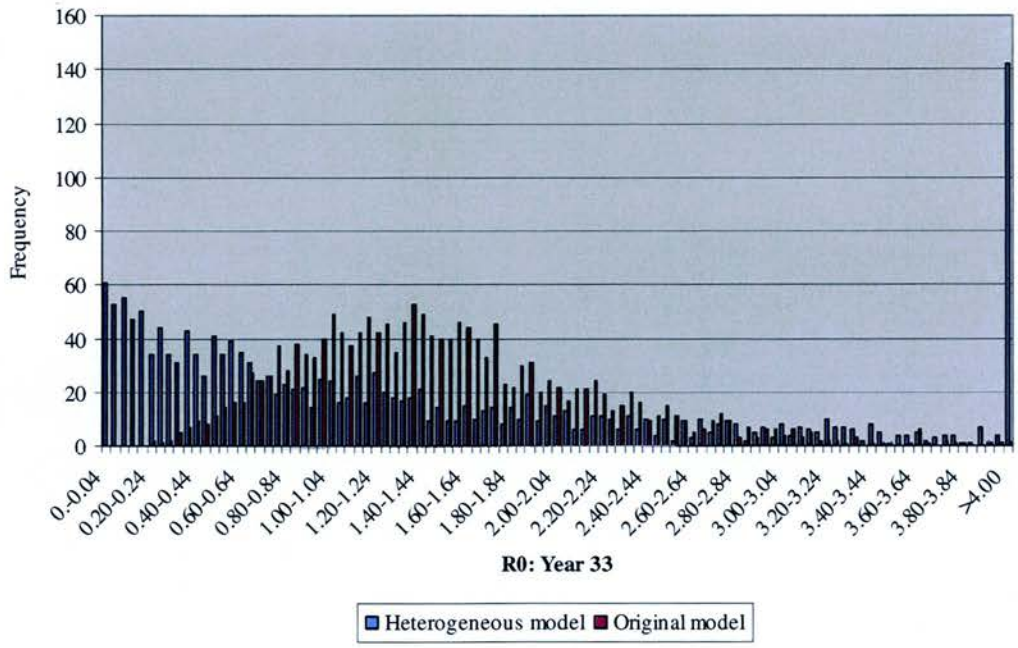
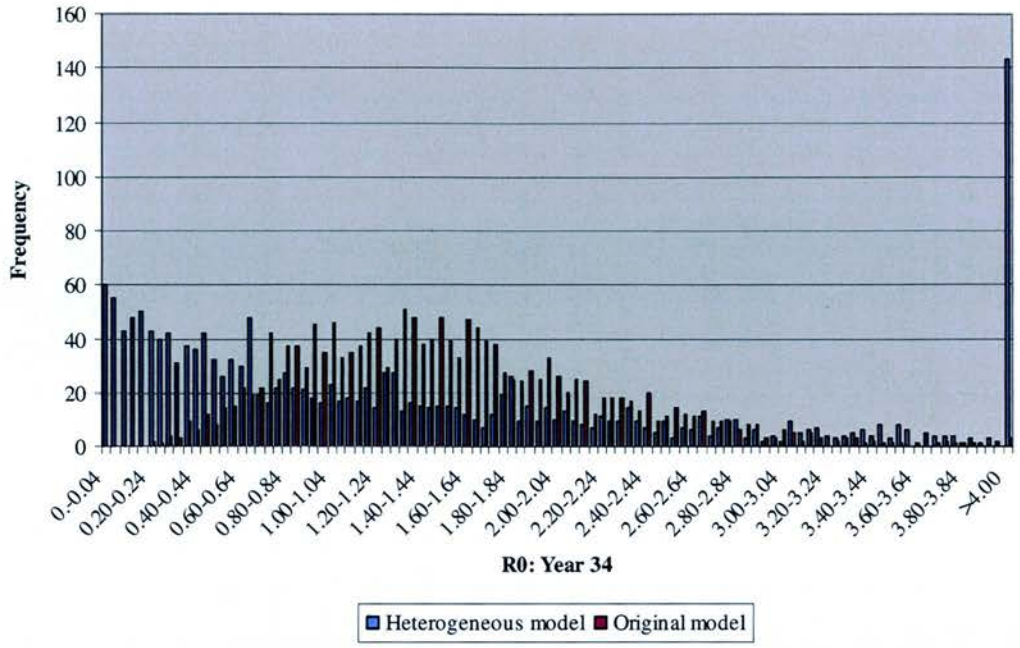
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 32
Heterogeneous model
Original model


160
140
120
100
80
60
40
20
0
Frequency
0,-0.04
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 33
Heterogeneous model
Original model


160
140
120
100
80
60
40
20
0
Frequency
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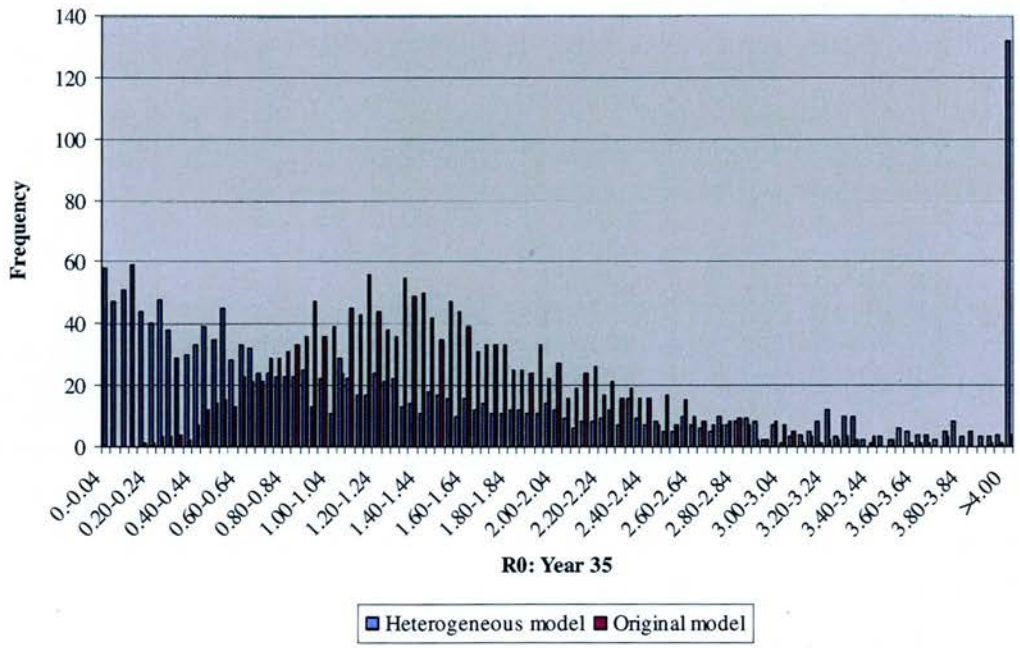
0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 34
Heterogeneous model
Original model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60
40
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0
Frequency
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0.20-0.24
0.40-0.44
0.60-0.64
0.80-0.84
1.00-1.04
1.20-1.24
1.40-1.44
1.60-1.64
1.80-1.84
2.00-2.04
2.20-2.24
2.40-2.44
2.60-2.64
2.80-2.84
3.00-3.04
3.20-3.24
3.40-3.44
3.60-3.64
3.80-3.84
>4.00
R0: Year 35
Heterogeneous model
Original model